Intake of dietary antioxidants and fatty acids as risk factors for asthma and reduced lung function in Chile

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INTAKE OF DIETARY ANTIOXIDANTS AND FATTY ACIDS AS RISK FACTORS FOR ASTHMA AND REDUCED LUNG FUNCTION IN CHILE

THESIS

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DEGREE

of

DOCTOR OF PHILOSOPHY

by

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Abstract

Background and aim: Oxidative stress is thought to play a central role in asthma. A decrease in dietary antioxidants intake has been suggested to play a part in the increase in prevalence of asthma in developed countries, but little information is available from developing countries. This thesis was aimed to assess the relation between intake of dietary antioxidants, fatty acids, and biomarkers of oxidative stress and of antioxidant status, and asthma symptoms, bronchial responsiveness (BHR), and lung function in young adults.

Methods: 1,192 adults born between 1974 and 1978 in Limache, a semi-rural area of Chile, participated in the study. They responded to a Spanish version of the European Community Health Research Survey questionnaire, were skin tested to eight allergens, and challenged with methacholine to assess BHR. Dietary intake was assessed with a food frequency questionnaire. Plasma levels of F2-isoprostanes, protein carbonyls, uric acid, and ferric reducing ability of plasma were assessed in half of the subjects.

Results: The large majority of dietary antioxidants tested, including grouped fruits, vegetables, nutrients and flavonoids were unrelated to respiratory symptoms of asthma and BHR, or to measures of lung function. BHR slope (mg^{-1}) was negatively associated with per-quintile intake of vitamin C (mg) (difference of means -0.35, 95% CI: -0.67 to –0.04). There was some evidence for a positive association between a greater FEV_1 (L) with total catechins (difference of means 0.04 L/ highest versus lowest quintile of intake (mg), 95% CI: -0.03 to 0.11).

Conclusion: This study provided little evidence of a protective effect of antioxidants on asthma and lung function, except for a marginal association between vitamin C and BHR, as well as between intake of catechins and lung function. Plasma biomarkers are unrelated to asthma in the general population.

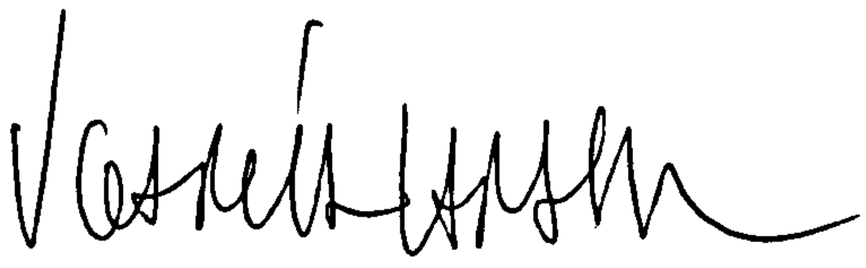
I declare that the work presented in this thesis is my own, and that no part has been submitted for a degree or comparable award of this or any other university or institution.

Work related to the methodology utilised in this thesis has been published previously, in a different form, as:

- García V, Rona R, Chinn S. Effect of the choice of food composition table on nutrient estimates: a comparison between the British and American (Chilean) tables. Public Health Nutrition 2004; 7: 577-83.

and

- García V, Arts ICW, Sterne JAC, Thompson RL, Shaheen SO. Dietary intake of flavonoids and asthma in adults. Eur Resp J 2005; 26: 449-452.



Vanessa García Larsen

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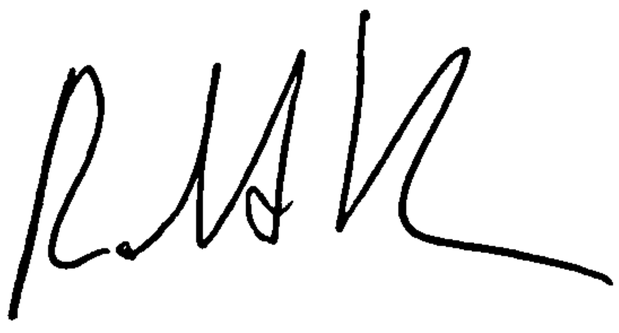
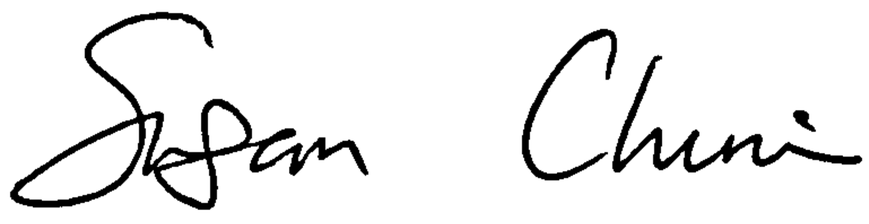


Professor Roberto Rona



Professor Susan Chinn

February 2006

Statement on the role of the candidate and other researchers in the design and development of the project

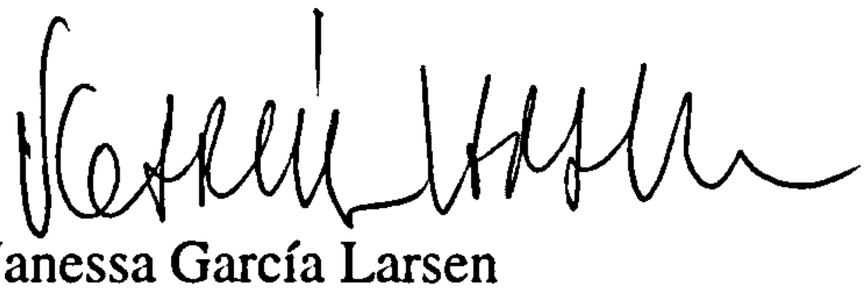
This thesis was part of a larger project aimed to explore risk factors of asthma and poor lung function in young adults from Chile. These risk factors included information on early childhood as well as on adult life. The role of dietary intake of antioxidants was considered in the application to the Wellcome Trust Fund as one of the risk factors of interest.

I was responsible for the design of the food frequency questionnaire (FFQ) administered to the participants. The criteria for designing such FFQ were to estimate the usual dietary intake as well as the specific intake of antioxidants of the subjects studied. Once the FFQ was designed, I was responsible for carrying out a pilot study in a sub-sample of the participants and training the nurses in charge of the fieldwork in how to administer the FFQ. Once in London, I regularly received FFQs for their revision and gave feedback to the fieldworkers when necessary.

I participated in the initial stage of the fieldwork, by visiting participants in their homes, inviting them to collaborate in the study, and administering the main questionnaire of the European Community Respiratory Health Survey (ECRHS) and the FFQ. I was also involved in the selection of the plasma biomarkers included in this thesis. Previously, during my Masters Degree in Chile, I learned the techniques to assess these biomarkers in a laboratory of the Faculty of Medicine, University of Chile under the supervision of Dr. Ramon Rodrigo. The technique of isoprostanes was new in this laboratory and I learned its procedure before coming to King's. As explained further in a separate statement, I was responsible for analyses and writing of two papers related to this PhD.

Professor Roberto Rona was responsible for the idea and design for the main study, as well as for the application for a grant from The Wellcome Trust, and has major responsibility for the analyses and publication of the results of the main project.

Drs. Patricia Bustos and Hugo Amigo in the University of Chile were responsible for coordinating and supervising the fieldwork of the project. Dr. Rodrigo was responsible for the chemical measurements of biomarkers in plasma.



Vanessa García Larsen

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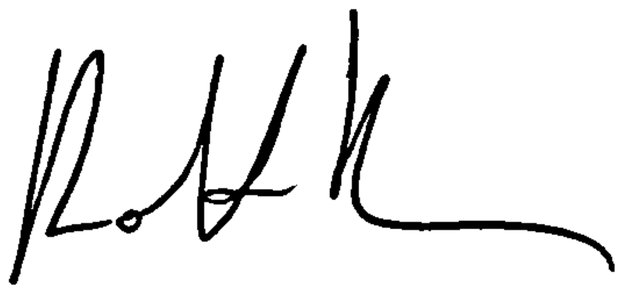
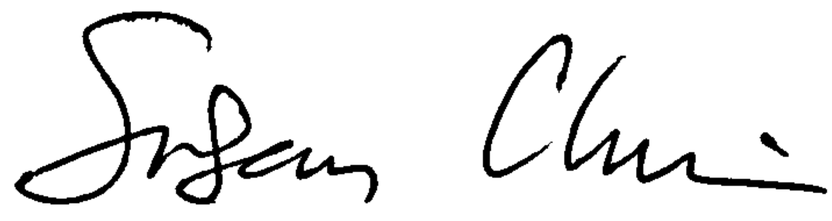


Professor Roberto Rona



Professor Susan Chinn

February 2006

Statement concerning conjoint work on published papers

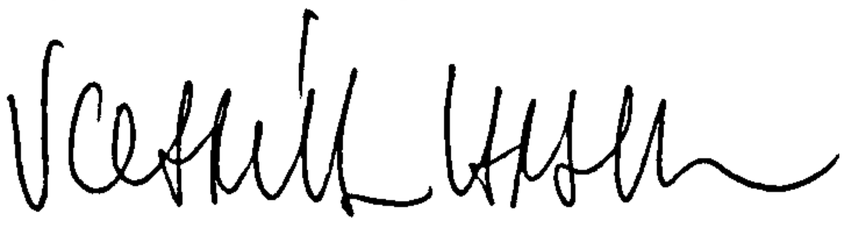
Candidate name: **Vanessa García Larsen**

Thesis title: **Intake of antioxidants and fatty acids as risk factors for asthma and reduced lung function in Chile.**

García V, Arts ICW, Sterne JAC, Thompson RL, Shaheen SO. Dietary intake of flavonoids and asthma in adults. Eur Resp J 2005; 26: 449-452.

The work for the paper above was carried out in collaboration with Dr. Seif Shaheen from the Department of Public Health Sciences, King's College London, Dr. Ilja Arts from the Institute of Food Safety, Wageningen University and Research Centre (RIKILT), Wageningen, The Netherlands, Dr. Jonathan Sterne from the Department of Social Medicine, University of Bristol, and Dr. Rachel Thompson from the Institute of Human Nutrition, University of Southampton. The analysis was planned as a learning experience to assess the intake of flavonoids in a population before the Chilean data collection was completed. We did not find associations between the intake of the three major classes of flavonoids investigated and asthma or chronic sputum in the study based on the FLAG survey led by Dr Shaheen.

In agreement with Dr. Arts, I used the flavonoid content database to analyse the dietary intake of these antioxidants in my PhD. The approach followed in the paper was helpful in the analyses I carried out in my PhD thesis.



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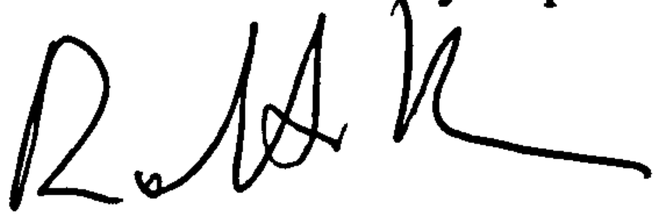

Professor Roberto Rona

Professor Susan Chinn

February 2006

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LIST OF ABBREVIATIONS

Acronym	Definition
•OH	Hydroxyl radical
AA	Arachidonic acid
ALA	3-alpha-linolenic acid
BHR	Bronchial responsiveness
CAT	Catalase
CO_2	Dioxide Carbone
COPD	Chronic obstructive pulmonary disease
COX	Cyclooxigenase
CI	Confidence interval
ECRHS	European Community Respiratory Health Survey
EPA	Eicosapentanoic acid
F2-ip	Isoprostanes (8-iso-PGF-$_{2\alpha}$)
$FEF_{25-75\%}$	Forced expiratory flow rate between 25 and 75% of FVC
FEV_1	Forced expiratory volume in 1 second
FEV_1%	FEV_1 as percentage of predicted value
FFQ	Food frequency questionnaire
FRAP	Ferric reducing ability of plasma
FVC	Forced vital capacity
FVC%	FVC as percentage of predicted value
g/d	Grams per day
GINA	Global Initiative on Asthma
GSH	Reduced glutathione
GSH-Px	Glutathione peroxidase
HDL	High density lipoprotein
H_2O_2	Hydrogen peroxide
ICC	Intraclass correlation coefficient
IgE	Immunoglobuline E
IL	Interleukine
IQR	Inter-quartile range
ISAAC	International Study of Asthma and Allergies in Childhood

List of abbreviations (continued)

Acronym	Definition
IUATLD	International Union Against Tuberculosis and Lung Diseases
LA	Linoleic acid
LOX	5-lipo-oxygenase
mg/d	Milligrams per day
MUFA	Monounsaturated fatty acids
MDA	Malondialdehyde
NO	Nitric oxide
$O_2^{\bullet-}$	Superoxide radical
O_2	Oxygen
OR	Odds ratio
PC_{20}	Concentration required to produce a 20% fall in FEV_1
PD_{20}	Dose required to produce a 20% fall in FEV_1
PEF	Peak expiratory flow
PUFA	Polyunsaturated fatty acids
RCT	Randomised controlled trial
ROS	Reactive oxygen species
RR	Relative risk
SD	Standard deviation
SE	Standard error
SFA	Saturated fatty acids
SOD	Superoxide dismutase
TEI	Total energy intake
TBARS	Thiobarbituric acid-reactive species

ACKNOWLEDGEMENTS

I am very grateful to the supervisors of my PhD, Professors Roberto Rona and Sue Chinn for their support, guidance and advice. I have been privileged to have the opportunity of working with them.

I am also grateful to the team of co-researchers in Chile led by Drs. Hugo Amigo and Patricia Bustos for their participation in the coordination of the fieldwork and collection of the data. This PhD would have not been possible without the *President of the Republic Scholarship,* which I was awarded by the Chilean Ministry of Planning, as well as The Wellcome Trust's funding of the main project on which this thesis was based. I am indebted to the staff at the Department of Public Health Sciences, King's College London, led by Professor Peter Burney. In particular, I have greatly enjoyed being part of the Asthma Group of researchers from whom I have received important feedback and advice. I would like to extend my thanks to The International Students House Trust for their support during my stay as a postgraduate student in London.

I am very grateful to Professor Dr. Iván Serra, whose dedication to public health and permanent trust in my abilities were an important encouragement to my pursuance of this PhD.

I would like to dedicate this work to my family in Chile. I am grateful for their emotional support and care throughout these years.

CHAPTER 1

Introduction: The relationship between diet and asthma

Asthma is a chronic disease highly prevalent in developed and some developing countries. Several aetiological risk factors have been identified for this disease, including genetic and environmental influences. Due to the rapid increase in the prevalence of asthma observed over the past three decades, it can be hypothesised that lifestyle-related to health and environmental factors play a role in the susceptibility of individuals. Dietary antioxidants are thought to be protective factors against the development of the disease. In this chapter the main environmental factors thought to play a role in asthma are described (Section 1.1). In section 1.2, the role that diet, and in particular antioxidants, have against asthma is illustrated as well as the epidemiological tools used to measure asthma and diet. The Chilean perspective in relation to diet and asthma is outlined in section 1.3. The specific objectives and the structure of this thesis are stated in Section 1.4.

1.1 Overview of the burden of asthma and its risk factors

Different definitions have been proposed for asthma. The common feature of asthma included in definitions is a chronic inflammatory condition in the airways with variable degree of obstruction and increased responsiveness to a number of factors [1, 2]. Over the last two decades, there has been a growing interest in this complex disease, for which increasing rates of prevalence have been reported, especially in developed countries [3-5].

To date, most of the epidemiological evidence on the burden of asthma comes from developed populations. Estimates of prevalence in developing countries are scarce. The available data from Central and South American countries largely comes from children and suggests that the prevalence is similar to that observed in developed regions and can be as high as that found in countries reporting the highest rates in the world, such as the UK, Australia and New Zealand [6, 7]. In Chile, a developing country with an emerging economy, the prevalence of asthma in children is as high as that reported in industrialised nations [8, 9]. There is some evidence that asthma morbidity is an important component of total chronic respiratory diseases in the country, as gathered

from medical surveys of Chilean families [10]. However, there are no comparable data on the prevalence and aetiological factors of asthma in adults using standardised epidemiological instruments.

In spite of the epidemiological evidence for an increase in the prevalence of asthma in many countries, the causes of the increase are still debated. It has been postulated that asthmatic subjects may have a genetic predisposition to develop the disease [11]. Elements of the pathogenesis of asthma, including the immune response and the regulation of pro-inflammatory cytokines, are also at least partly under genetic control and are activated under environmental factors in genetically predisposed subjects [12, 13].

The rapid increase observed in asthma prevalence cannot be explained on the basis of genetic predisposition alone. Therefore, attention has been centred on a number of environmental factors to which such an increase could be attributed. Indoor and outdoor allergens, such as domestic mites, animal allergens, pollens, fungi and molds, have been suggested to have a role in the manifestation and persistence of asthma [14, 15]. Environmental pollutants, mainly industrial smog and those derived from ozone and nitrogen oxides, may intensify clinical manifestations of asthmatic subjects [2].

The overall magnitude of the contribution of these factors in relation to the rapid increase of asthma is under debate. Although allergens seem to be strongly related to the prevalence of asthma, it does not appear that there has been a significant increase in population exposure to domestic allergens, including that to house dust mite [16]. In addition, air pollution has decreased in countries where an increase in asthma has been observed, including those of Western Europe, USA, Australia, and New Zealand. Furthermore, the worldwide pattern of air pollution and asthma distribution does not support air pollution as a major causative factor for asthma.

Developed countries with a higher socio-economic level have the highest prevalence of asthma. It has been suggested that better hygienic conditions derived from this affluent status may be in part related to the increase in allergic diseases including asthma [17]. One of the underlying mechanisms hypothesised for the rise of atopy and asthma in industrialised countries, is the decrease in the incidence of early childhood infections,

and the consequent expansion of T helper type 2 lymphocytes (Th2), which would lead to an imbalance in the regulatory mechanisms of the inflammatory response later on in life [18].

Children with siblings are thought to be more likely to acquire infections during their childhood and therefore would be protected against allergic diseases during adult life [19-21]. This observation has contributed to the hypothesis that family size and, in particular number of older siblings may be related to asthma. The evidence for this has emerged mainly since the 1990s [22], and changes in family size over the past 30 years do not appear to explain the increase in asthma observed in the same period in the United Kingdom or New Zealand, two of the countries with highest prevalence [23].

1.2 The relationship between diet and asthma in the epidemiological setting

Dietary intake is another factor postulated to play a role in the development of asthma, and possibly associated with the increased prevalence of this disease in the last three decades. Over this period, the dietary pattern in many developed and developing societies has changed. The current high prevalence of asthma may be related to a diet characterised by lower consumption of fresh fruits, vegetables and fish, and high consumption of foods rich in saturated fatty acids, sugars, and salt [24].

One of the earliest hypotheses that changes in diet may be related to changes in prevalence of asthma was postulated in the 1980s when Burney observed that regional variations in mortality for asthma in England and Wales were related to purchase of table salt [25]. In 1994, Seaton first hypothesised that an insufficient intake of dietary antioxidants could be related to asthma [26]. Since then, several researchers have proposed that diet may have an impact on either the aetiology or severity of asthma [27, 28].

Ecological studies suggest that changes in diet related to a decrease in consumption of dietary sources of antioxidants, as well as variation in the type of fatty acids consumed, may be contributing to the increase in the prevalence of asthma [28-30]. This hypothesis arises from the fact that the inflammatory-immune process that characterises asthma involves the permanent production of a series of molecules with

oxidative capacity, known as reactive oxidative species (ROS) [31], which if not contained could lead to a chain of molecular events that may end in the worsening of the asthmatic response [32].

A higher intake of specific dietary antioxidants has been suggested as a protective factor by preventing, or at least modulating, the damage that ROS generate, thus attenuating the manifestation of asthma symptoms. In addition, dietary antioxidants have anti-inflammatory properties, as they directly prevent the activation of inflammatory cells [29]. Another component of the diet, polyunsaturated fatty acids (PUFA), play a key role in the inflammatory response. When they are oxidized, the synthesis of a number of pro-inflammatory molecules takes place, directly stimulating the progression of the asthmatic response [32]. Therefore, these fatty acids could also have a protective effect.

The largest epidemiological evidence of an association between dietary antioxidants, PUFA and asthma comes from cross-sectional studies, with some evidence from randomised controlled trials (RCT) and longitudinal studies that have tested the hypothesis that dietary intake may affect the incidence of asthma [27-30]. Symptoms of asthma in epidemiological studies have been mainly ascertained by questions on respiratory symptoms, and more recently have included measurement of BHR. The use of standardised questionnaires on asthma in children [33] and adults [34] has facilitated the comparison of prevalence of the disease and its symptoms, defining asthma based on questions of personal perception of symptoms, which sometimes has the limitation of different interpretations given to these questions, due to cultural bias.

Dietary questionnaires have been the main tool utilised to assess intake of antioxidants, with the limitation that they may not measure endogenous levels of antioxidants that are effectively absorbed or the effects that antioxidants may or may not exert against oxidative damage in the lungs. In spite of these limitations, dietary questionnaires can still offer a reliable estimate of the dietary intake in epidemiological settings, especially if its validation and reproducibility have been tested, and if it has been carefully designed to meet the aims of the research [35, 36]. A number of measurements in blood of biomarkers have been included in epidemiological studies as complementary tools to the information given by dietary questionnaires.

Generally, a biomarker is defined as a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacological responses to a therapeutic intervention [37]. Nutritional biomarkers can provide an estimate of nutrient intake and nutritional status, independent of the information given by individuals, allowing for more accurate information of the endogenous antioxidant/oxidant status [28, 29]. The main limitation is the high cost and complexity that may involve their measurement.

In the study of asthma, two main groups of biomarkers have been used to assess the relationship with diet. These are biomarkers of antioxidant status/intake, through the assessment of antioxidant enzymes or nutrients in plasma, and biomarkers of oxidative stress, where oxidation of molecules such as fatty acids and proteins is assessed [38, 39].

Overall, there are several tools to evaluate the possible association between diet and asthma in epidemiological studies. A combination contributes to obtaining more detailed estimates of the nutritional status of an individual and the relationship with asthma.

1.3 The Chilean perspective

In spite of the increasing epidemiological evidence reported in relation to asthma and diet little information has been provided regarding dietary intake pattern as a risk factor for asthma in the Chilean population, a country with a similar prevalence in children to that reported for developed countries [6, 8, 9]. In addition, there is little known about the prevalence of asthma and the possible aetiological factors responsible for the disease in adult population.

The dietary pattern observed in the country has followed the same evolution as that described for America and Western European countries, with an insufficient intake of fruits and vegetables and an increase in the intake of saturated fatty acids and vegetable oils [40]. This has been mirrored in a concomitant increase in the figures of obesity, which currently affects nearly 20% of the population [40].

1.4 HYPOTHESES, AIMS, AND STRUCTURE OF THE THESIS

The primary hypothesis of this thesis is that intake of specific dietary antioxidants is associated with asthma in a population of Chilean young adults. As an approach to define asthma, self-reported respiratory symptoms and bronchial responsiveness (BHR) were determined. The main contenders for a beneficial effect should be dietary antioxidants with known antioxidant properties that the literature suggests might decrease the risk of asthma.

To address this hypothesis, the specific aims are to:

1 Explore the associations between self-reported asthma symptoms and BHR, with dietary intake of:
   a. Fruits and vegetables rich in antioxidants; antioxidant vitamins C, E and total vitamin A
   b. Minerals selenium and zinc and flavonoids
   c. Omega 3 fatty acids, and the ratio omega 6/omega 3 (ratio n6/n3)

A second hypothesis is that biomarkers of antioxidant status and of oxidative stress in plasma would reflect to some extent the association between oxidative stress and asthma. For this purpose, the following objectives are to be addressed:

1. Evaluate the association between asthma symptoms and BHR, with antioxidant status assessed through plasma levels of ferric reducing ability of plasma (FRAP) and uric acid.
2. Assess the association between asthma symptoms and BHR with oxidative stress as measured through the plasma levels of protein carbonyls and F2-Isoprostanes (F2-ip).
3. Assess the correlation between biomarkers of oxidative stress and of antioxidant status with dietary antioxidants and food items.

A third hypothesis in this thesis is that intake of dietary antioxidants is associated with a better lung function in young adults, as well as with lower levels of oxidative stress. For this purpose, the aims are to:

1 Explore the associations between FEV_1 and the ratio FEV_1/FVC with:
   a. Fruits and vegetables rich in antioxidants
   b. Antioxidant vitamins C, E and total vitamin A; minerals selenium and zinc and flavonoids
   c. Omega 3 fatty acids, and the ratio omega 6/omega 3 (ratio n6/n3)
   d. Biomarkers of antioxidant status and oxidative stress in plasma

This thesis is based on a prospective study aimed to assess current and early risk factors on asthma in young adults born between 1974 and 1978 in Limache and its vicinity. This study reports a cross-sectionally designed study looking at the dietary intake of this population as a risk factor for asthma.

This thesis is focused on the study of dietary intake as a risk factor for asthma in young adults of Chile, a middle-income industrial country. The high rates of asthma in Latin America may be similar to those observed in developed countries [41], but they may not have the same risk factors and its study may provide epidemiological clues in other settings, a need that has been expressed by researchers from developed societies [42].

The evidence on the relationship between diet and asthma is scant in Latin America. There is some indication that supplementation with antioxidant enzymes may protect against moderate or severe asthma in Mexican children living in areas with high ozone levels [43], but no studies have been carried out in other countries of this region. Similarly, no attempts have been made to evaluate the association between dietary intake and asthma in children or adults from South America. Food habits may differ greatly from one country to another and local factors may influence the effect of food intake on asthma.

The inclusion in this study of assessment of plasma biomarkers of antioxidant status aims to confer more certainty on the relationship, if any, that exists between oxidative stress that is present in young adults with asthma symptoms. The assessment of

oxidation of lipids and proteins in a population-based study is novel. Although these biomarkers have been increasingly used with asthmatics in clinical studies, these normally include a small number of participants, and scarce evidence has been provided on their applicability in studies on general population.

In Chapter 2 the main components of the definition of asthma as used in epidemiological studies are described. A brief description is given of the molecular pathogenesis of the disease, which is characterised by an inflammatory process mediated by oxidative stress. An overview of the prevalence is also presented in order to give a picture of the magnitude of the disease in developed societies with a similar epidemiological profile to Chile.

The current knowledge on the mechanisms by which dietary antioxidants are involved in asthma is described in Chapter 3. This is covered in two sections, one describing the molecular role that these antioxidants have in the pathways of asthma, while the second comprises a review of the epidemiological evidence that has been gathered so far for each of these antioxidants and asthma. This thesis is concerned with the situation regarding dietary intake and asthma in adults so the review is limited mainly to this group of people, but a brief overview in children is also provided.

The role of biomarkers as tools in the assessment of oxidative stress and of antioxidant defences against asthma is reviewed in Chapter 4. A description of those included in this study is given, and others commonly used in other studies but unfeasible to include in this thesis, are also described.

The methodologies used for the study are described in Chapter 5. The main questionnaire utilised to assess asthma was obtained from the European Community Respiratory Health Survey (ECRHS) using the Spanish version adapted to the local lexicon. The results are presented in Chapters 6 to 8. In Chapter 6 the general findings on prevalence of symptoms and respiratory outcomes are described, as well as results for dietary intake. In Chapter 7, the associations between respiratory symptoms and BHR with diet, flavonoids and biomarkers are presented. In Chapter 8, the association between best FEV_1, as well as the ratio FEV_1/FVC with dietary intake of antioxidants, flavonoids and biomarkers is described. In Chapter 9 the results of the thesis are

summarised and discussed and their contribution to the current knowledge on dietary intake and asthma is presented.

CHAPTER 2

The components of asthma and the magnitude of the problem

This chapter provides a definition of asthma (Section 2.1) and the elements that comprise the definition used for epidemiological purposes (Section 2.2). In section 2.3 the pathogenesis of the disease is outlined. A description of BHR as a more specific measurement of asthma is provided in section 2.4, as well as the definition of lung function, an indicator of chronic respiratory disease. The last part of the chapter summarises the international prevalence of asthma (Section 2.5).

2.1 Definition of asthma

Asthma is one of the commonest illnesses that affects people across the world. In spite of this, there is not a universally accepted definition for asthma [44]. The World Health Organization defined asthma in 1975 as 'a chronic condition characterised by recurrent bronchospasm resulting from a tendency to develop reversible narrowing of the airways lumina in response to stimuli of a level or intensity not inducing such narrowing in most individuals' [45].

The American Thoracic Society emphasises that the physiological manifestation of asthma is characterised by one or more of the following alterations of respiratory function: (1) diminished vital capacity, forced expiratory flow rate, and maximal voluntary ventilation; (2) increased airway resistance; (3) increased residual lung volume; (4) abnormal intrapulmonary gas mixing; and (5) hypoxemia and hypercarbia. According to the ATS, "the degree of functional alteration depends on the severity of the bronchial obstruction" [1].

The Global Initiative on Asthma (GINA) adds that it is an "inflammatory disorder of eosinophils and T lymphocytes, causing in susceptible individuals breathlessness, chest tightness and cough, particularly at night and/or early morning". These symptoms are usually associated with widespread but variable airflow limitation that is at least partly reversible either spontaneously or with treatment" [2].

2.2 EPIDEMIOLOGICAL DEFINITION OF ASTHMA

The majority of epidemiological studies have used three main methods to classify individuals as having asthma. These are (1) a positive answer to a question on whether a person has asthma, usually diagnosed by a doctor; (2) a positive answer to whether he/she has a symptom of asthma, usually wheeze; and (3) a measurement of increased BHR.

Questionnaires are regarded as the main tool to obtain information on asthma symptoms in the population. Therefore, the diagnosis of asthma has largely relied on the answers provided by the subjects themselves, or by parents in the case of children [46]. The commonest questions included in a questionnaire are whether the participant has ever been diagnosed as having asthma, and the presence of symptoms like wheezing or breathlessness [47-49]. A main limitation arises, as the words wheezing, chest tightness and breathlessness may mean different things to different people within and between societies, therefore leading to bias in comparisons.

The use of questionnaires has as advantages that they have a relatively low cost and can cover a large population in a relatively short period of time. A large number of questionnaires can be administered under different conditions, such as person-to-person interview, self-administered, or by telephone, saving time and human resources. In developed societies, a postal questionnaire has been widely used, with variables rates of response. However, this alternative is not always feasible in countries with high or partial levels of illiteracy or unreliable postal services.

The ECRHS, carried out to assess the prevalence of asthma across Europe, included both validated questionnaires and objective measurements. Since the publication of its protocol in 1994, it has produced data on prevalence of asthma symptoms in adults aged 20 to 44 years old. This survey aimed to estimate the variation in the prevalence of asthma, and symptoms of asthma across European countries. It also aimed to estimate the variation in the exposure to several environmental risk factors and their association with asthma, and the extent to which they may explain the variation in asthma found between centres in the study.

The main questionnaire included a number of questions on symptoms of asthma and medical history, as taken from The International Union Against Tuberculosis and Lung Disease (IUATLD) questionnaire [34]. It also included questions on social status, occupation, smoking, housing conditions, and medication. In addition to this questionnaire, measurements of airway obstruction (lung function and responsiveness to methacholine) and atopic status (skin prick test and serum IgE) were included in the survey.

The International Study of Asthma and Allergies in Childhood (ISAAC) was the first study assessing variations of prevalence in children between countries. As with the ECRHS, ISAAC aimed to assess prevalence and its risk factors through the use of a questionnaire. It also aimed to describe the severity of asthma, rhinitis and eczema in children, and provided baseline measures for assessment of future trends in prevalence, thus allowing for comparisons in change of prevalence across time. The survey included children aged 6 to 7 years old with questions completed by parents and 13 to 14 years old who responded to a self-administered questionnaire at school. They were also shown a video picturing asthma symptoms after which the children answered several questions related to it.

The survey has been carried in most of the continents, being considered the most extensive international survey on prevalence of asthma symptoms so far carried out.

These two surveys have provided insights in the variations of prevalence of asthma symptoms, both in adults and in children. A study comparing the level of agreement and correlation between the ECRHS and ISAAC projects showed a strong correlation between the prevalence reported by the two surveys, with generally good agreement between them [50].

2.3 Pathogenesis of asthma

The central feature in asthma is airway inflammation. Several pathways lead to the inflammatory response that will cause the manifestations of asthma symptoms [51]. One of them is represented by the role of leukotrienes. They are synthesised from arachidonic acid (AA) released from the cell membranes. Once this occurs, AA can be

metabolised through two enzymatic pathways. One is through cyclooxigenase (COX), which will lead to formation of prostaglandins, thromboxanes and prostacyclines.

The second is through lipoxigenase (LOX), which will lead to formation of leukotrienes. The first leukotriene synthesised is LA_4, considered the precursor of all the other families of leukotrienes. Other leukotrienes are also generated, such as LB_4, LTC_4, LD_4 and LE_4 under the regulation of several enzymes. It has been established that LB_4 is a potent stimulus for activation of leucocytes, stimulates the secretion of a ROS, superoxide anion and can affect the synthesis of IgE. LC_4, LD_4 and LE_4 are considered the most potent inducers of bronchoconstriction, related to the functions of smooth muscle contractility and vascular permeability [51].

The other main pathway involved in the inflammatory response is that related to the immune system, in which lymphocytes and cytokines play a major role. The series of events start when T lymphocytes are presented with an antigen by macrophages. This results in the production of two subtypes of lymphocytes, namely T helper 1 (Th1) and T helper 2 (Th2). They produce several cytokines, which will activate a series of mediators of inflammation. Interleukines (IL) 4 and 13 are the two cytokines suggested to play a central role, as they regulate the synthesis of IgE [52]. IgE binding to allergen can link the high-affinity IgE receptor on mast cells, resulting in release of mediators that include histamine, prostaglandins, leukotrienes and enzymes [52, 53].

IL-4 is also involved in the activation of mast cells jointly with IL-9, while IL-3 activates eosinophils. The sputum of asthmatic patients contains large numbers of eosinophils [54], which are considered pivotal cellular mediators of asthma. The release of these substances leads to BHR and airway obstruction, and the manifestation of clinical symptoms. These include, to different extent of intensity and reversibility, wheezing, cough early in the morning, tightness of chest, waking up with cough, and attacks of asthma.

IgE also plays a role in the manifestation of asthma. The development of atopy is associated with an increased persistence and severity of asthma. Atopy is defined as an abnormally high production of IgE making individuals susceptible to develop allergic reaction to several environmental factors. This alteration in the production of IgE has

been considered an important component of asthma, as it is present in a large number of asthmatic subjects [55].

2.4 ASSESSMENT OF LUNG FUNCTION AND AIRWAYS OBSTRUCTION.

2.4.1 Lung function and its measurement

Lung function can be measured using spirometry. Trained professionals, who need patient co-operation to produce a valid result, usually carry them out [56].

The volume of air expired with each normal respiration constitutes the tidal volume (Vt), and can be reduced in painful or limiting processes of the bones or muscles involved in respiration [57]. The volume of air left in the lungs after a normal expiration represents the functional residual capacity (FRC); the volume expired in a forced expiration starting at a normal inspiration is the expiratory reserve volume (ERV). The volume expired from full inspiration (total lung capacity (TLC)) to full expiration (residual volume (RV)) regardless of time, is the vital capacity (VC) [58].

Dynamic lung function can be explained as the changes in volume *vs.* time (flow); the volume exhaled from full inspiration to full expiration under forced expiration constitutes the forced vital capacity (FVC) [59]. The volume expired in the first second of forced expiration is the forced expiratory volume in 1 second (FEV_1). Maximal mid expiratory flow ($FEF_{25-75\%}$) represents the mean flow achieved during the middle of the FVC (in forced expiration manoeuvre from full inspiration between 25% and 75% of FVC) [60]. FEV_1 and FEV_{25-75}, and to a lesser extent FVC, are reduced during obstruction to airflow.

2.4.2 Bronchial Responsiveness

Bronchial responsiveness (BHR) can be defined as the tendency for the airways of asthmatic subjects to broncho-constrict when exposed to various chemical and physical stimuli [61]. Exposure to stimuli, such as allergens, which are specific for an individual, produce a different effect, in that the non-specific stimuli generally cause a short-lived period of broncho-constriction without inducing significant airway inflammation whilst antigenic stimuli cause more prolonged bronchoconstriction with

an immediate response lasting for 1-2 hours that may follow a late response at 4-8 hours, which is characterised by inflammatory cell recruitment to the airways [62]. Various broncho-constrictor stimuli can be used to measure the degree of BHR, including inhaled histamine or methacholine, inhaled hypertonic saline or distilled water, exercise or cold air [63, 64].

During obstructive processes, the reduction of FEV_1 is greater than the reduction of FVC and the FEV_1/FVC ratio is reduced [65]; conversely, in restrictive lung disease the reduction in FVC is greater than in FEV_1 and the ratio is increased or normal. In asthma, peak expiratory flow (PEF) variation is closely related to FEV_1 and can be used to monitor asthmatic episodes and treatment responses [66].

The assessment of BHR has been done mainly through challenge with histamine and more recently methacholine. The first occurs naturally in humans, while the second is artificial. The tests of BHR give a measure of the concentration (PC_{20}) or dose (PD_{20}) required to produce a 20% fall in FEV_1. When normal individuals are challenged with a non-specific stimulus there is usually a small degree of bronchoconstriction but the FEV_1 value reaches a plateau before a 20% fall is achieved [67]. In asthmatics, bronchoconstriction typically occurs at a much lower concentration and there is no plateau, so that increasing the histamine dose further produces greater bronchoconstriction [68].

Asthmatic subjects generally have a PC_{20} to methacholine or histamine less than 8mg/ml while most non-asthmatic subjects have a PC_{20} greater than 16 mg/ml. There is some overlap, and defining an exact level of BHR, which would distinguish asthmatic from non-asthmatic subjects is not possible. Evidence suggests that the BHR in population exhibits a continuous distribution, in which the majority of asthmatic subjects have values in one tail [69].

2.5 Prevalence of asthma

There is a strong body of evidence suggesting that asthma is highly prevalent in many countries and it has been increasing during the last decades [70, 71]. Its prevalence shows large variation across the world, being higher in countries with emerging or

advanced economies. The highest prevalence of asthma (> 20%) has been found in English speaking countries such as Australia [72], and New Zealand [73], while low rates have been reported in Eastern European countries (5-15%). Countries with the lowest reported prevalence of asthma (< 3%) include South Korea, Russia, Uzbekistan, Indonesia and Albania [74].

The epidemiological evidence of prevalence in Latin America is scant and comes mainly from studies in children [33]. The prevalence of a number of asthma symptoms shows variations across the countries. Wheeze in the last 12 months varies from 6.8% (Punta Arenas, south of Chile) to 26.0% (Lima, Peru) in children aged 13-14 years old, with an overall rate of prevalence of 16.9%. Similarly, variations from 6.6% (Argentina) to 28.0% (Peru) have been reported for those ever having asthma, with an overall rate of 13.4%. In children aged 6-7 years old wheezing in the last 12 months is present in a range of 8.6% (Cuernavaca, Mexico) to 32.1% (Costa Rica). 'Ever had asthma' has been found in similar proportions to these other symptoms, with an overall rate of 12.4%.

Some researchers have suggested that that there has been an over-reporting of asthma, so the increase in its prevalence is only apparent in developed countries [75, 76]. They believe that between 1970 and 1990 the labelling of wheezing changed in Britain and Australia, source of many papers published [77]. These authors suggested that surveys should include objective assessments of asthma, including measurement of lung function and BHR, which were introduced into surveys in the 1980s. These arguments now seem somewhat weak, as several large surveys have introduced the use of such instruments, confirming a high and increasing prevalence in children and young adults [71].

Conclusion

Asthma is a complex disease, which affects a large proportion of people worldwide. Its prevalence is higher in wealthier and more developed countries. The assessment of such prevalence has been obtained from questionnaires to elicit the symptoms that could be related to asthma. The inclusion of more objective measurements as indicators

of atopy or BHR are useful to increase certainty in the estimates of the prevalence and impact of the illness in the community.

In spite of the increasing efforts to describe the prevalence of asthma through standardised instruments in different countries, there is still uncertainty about the extent to which the prevalence differ in South American countries in comparison to other developing and developed countries.

The explanations for the magnitude of the prevalence of asthma worldwide are being centred in the contribution of several environmental factors. In the next chapter, the evidence accumulated in relation to dietary intake as a risk factor for asthma is described.

CHAPTER 3

Dietary antioxidants, fatty acids and asthma

This chapter summarises the relationship between diet and asthma derived from the epidemiological literature. The chapter is divided into three sections that describe the molecular roles and the epidemiological evidence by which oxidative stress in general and specific antioxidants or nutrients in particular are related to asthma.

In the first section of the chapter, the mechanisms by which oxidative stress is generated, particularly in asthmatic subjects, are described. There are a number of endogenous antioxidant defences available to constrain the oxidative damage that is generated, which are described in this section jointly with their biological role in the lung and potentially in asthma.

The second section describes the dietary antioxidants and minerals thought to play a part in lung function and asthma. The molecular mechanisms by which they may prevent or attenuate the oxidative damage caused in asthma are mentioned, followed by a literature review of the epidemiological evidence so far accumulated in relation to each antioxidant and lung function, and asthma, as obtained from studies looking at dietary intake, blood biomarkers of nutrient exposure, and RCT. The data reviewed are largely limited to adults, as this was the population investigated in the study for this thesis.

The third part of this chapter defines polyunsaturated fatty acids (PUFA) and their possible contribution to the pathogenesis of asthma, describing the biological mechanisms by which these nutrients are related to the inflammatory response of asthma. The epidemiological evidence obtained from observational studies and RCT is presented. Finally, conclusions are drawn from the available epidemiological evidence of diet and asthma.

3.1 OXIDATIVE STRESS AND DIETARY ANTIOXIDANTS: THE MOLECULAR BASIS

3.1.1. Reactive oxygen species (ROS) and oxidative stress

The epithelial lining of the respiratory system is highly vulnerable to oxidative damage, as a consequence of its large surface area and its role in gas exchanges and host defence [78]. The deleterious effect of oxygen, at least as it relates to free radical mechanisms, occurs directly through damaging the structure and functionality of cellular lipids, proteins, and nucleic acid or indirectly, through activation of inflammatory cells able to create additional free radicals. Existing oxidative stress appears to prompt the inflammatory response of asthma and also appears to be endogenously generated as a consequence of this response, therefore perpetuating the manifestation of inflammation [31, 79].

Oxidation happens in physiological conditions in all the biological systems, resulting in the production of small amounts of ROS [31]. When an imbalance is occurring in favour of the amount of ROS produced against the scavengers that contain them, a stage of oxidative stress takes place. During the inflammatory-immune response that characterises asthma, mast cells (macrophages, eosinophils and monocytes) are activated. Once this occurs, these cells are able to release high amounts of ROS, mainly hydrogen peroxide (H_2O_2), hydroxyl radical (•OH) and superoxide radical ($O_2^{\bullet-}$). Under conditions of chronic inflammation and tissue damage in the airways, high amounts of iron are also released. This metal can react with H_2O_2 in a reaction known as Fenton's reaction, resulting in the production of •OH [52].

Another group of cells that contribute to the propagation of ROS is that of pro-inflammatory cytokines activated during the asthmatic response [80]. They induce the production of a group of enzymes involved in the production of nitric oxide (NO), whose exaggerated production (as that occurring when inflammation is activated) facilitates its reaction with superoxide, resulting in the synthesis of peroxynitrite, a powerful oxidant able to react with several proteins and lipids leading to their oxidation [81].

The deleterious effects that ROS can generate include oxidation of PUFA, proteins and enzymes [82]. ROS are also capable of modulating the expression of a variety of immune and inflammatory molecules, perpetuating and exacerbating inflammation. PUFA are the molecules most vulnerable to be oxidised due to their large number of double binds. Thus, a large number of molecules of lipid peroxides could be produced if a single •OH attacks PUFA (located in the membrane cell). The process may continue as a chain reaction depending on the level of oxidative stress taking place. The peroxidation of PUFA generates the synthesis of pro-inflammatory compounds, thus worsening the asthma response [83].

The ROS can usually be contained by the action of several biological systems known as antioxidants, which act as 'scavengers' in the intra- and extra- cellular space, thus protecting the cells from radical-mediated damage. One of them consists of the antioxidant enzymes catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), which through a sequence of reactions transform ROS into water and other non-toxic molecules as final products. Another group is represented by antioxidant vitamins and minerals, which can also react with ROS, thus reducing them and preventing the damage to vulnerable molecules [31].

3.2 Dietary antioxidants and asthma

There are several antioxidants in the diet that represent the exogenous antioxidant defence against oxidative damage. They can be grouped into vitamins, minerals and polyphenols. Amongst the first group the most well recognized as antioxidants are: vitamin A and its precursors (i.e. α and β-carotene, lycopene, retinol), found in carrots, tomato and dark green leafy vegetables; vitamin C, abundant in citrus fruits; and vitamin E (γ-tocopherol), abundant in almonds, peanuts, sunflower seeds, and vegetable oils. Amongst minerals, selenium, zinc and to a lesser extent magnesium, widely distributed in red meat, seafood, dairy products and some vegetables, have been described as having antioxidant activity. Flavonoids, a family of polyphenols, are widely found in onion, apples, red wine and tea, and have been described as potent antioxidants [84].

3.2.1 Vitamins and the experimental evidence

Antioxidant vitamins are strong scavengers, reducing many of the circulating ROS. Vitamin E, a lipophilic molecule is concentrated inside the membranes and in blood lipoproteins. Its two main antioxidant actions are its capacity to act as a scavenger of •OH, (thus protecting membranes of oxidation) and its ability to act as a chain breaking antioxidant, by donating hydrogen to peroxy-radicals, making ROS and lipid peroxides less-reactive molecules. In the extra-cellular environment, vitamins C and β–carotene (a precursor of vitamin A) also act as potent scavengers [85].

These three antioxidant vitamins are directly involved with the prevention of oxidative damage in the lung. They are supporters of maintenance of the alveolar surfactant, whose integrity is a determinant of a normal lung function. It is estimated that approximately 90% of the alveolar surfactant corresponds to lipids (mainly PUFA and cholesterol) that are highly vulnerable to undergo oxidation due to the permanent interchange of O_2 and CO_2 that takes place in the alveolus. According to these findings β-carotene, vitamin C and E are closely involved with the prevention of lipid peroxidation and tissue damage [86, 87].

A specific group of alveolar cells, namely type II cells, are responsible for the synthesis of surfactant lipids and of the alveolar surfactant and its assemblage. Vitamin E appears to be one of the most important antioxidants at this level, preventing these lipids from oxidation. There is evidence that after being recruited by specific receptors from the HDL circulating in the interstitial tissue, vitamin E is secreted from type II cells jointly with the surfactant [86].

Vitamin A (retinol) is found in foods of animal origin such as meat, liver, eggs and dairy products. Vitamin A plays a central role in the development of respiratory epithelium, as it is involved in the synthesis of phospholipids of the alveolar surfactant, particularly in newborns [88]. At this stage, this vitamin is essential in the regulation of cellular differentiation of the respiratory epithelium and lung epithelium, therefore facilitating a normal respiratory epithelial differentiation, lung function and pulmonary immune function in later life [87].

In vitro studies on human lung cells have demonstrated that β–carotene, both separated and jointly with vitamins E and C, has antioxidant capacity [89]. This has been reflected in a lower production of products of oxidative stress (protein carbonyls and isoprostanes) in cells exposed to varying concentrations of vitamins and of O_2 when compared to cells that were not exposed to antioxidants [89]. This suggests that a deficient amount of these vitamins results in oxidative damage and ultimately in tissue damage in the airways. Kelly *et al.* tested this hypothesis in a clinical study carried out in mild asthmatic subjects, finding that asthmatics had significantly lower concentrations of vitamins C and E in bronchoalveolar lavage compared to healthy controls [90].

The beneficial relationship between antioxidants and containment of the oxidative damage that takes place in the pathophysiology of asthma has motivated an increasing interest into whether these associations could be seen in epidemiological studies.

3.2.2. Vitamins and the epidemiological evidence

Vitamins C, E, A and β–carotene are the antioxidant vitamins most widely investigated in relation to their effect on lung function and asthma symptoms. The epidemiological evidence on the association between lung function, symptoms of asthma, BHR and atopy is presented for each vitamin, grouping studies according to the respiratory outcome studied and to whether vitamins were studied from dietary intake or blood levels.

3.2.2.1 Vitamin C and lung function

The association between dietary intake of vitamin C and lung function has been extensively investigated in cross-sectional studies. A summary of their main results is presented in Table 3.1. These surveys were large population-based studies in adults, that obtained information about dietary intake of vitamin C from self-administered dietary questionnaires. Most of them assessed lung function based on net changes in FEV_1 and FVC, the exception being Schunemann *et al.* who analysed these outcomes as percentages of predicted values [91].

In 1995, Britton *et al.* reported a positive association between a 1 SD increase in intake of vitamin C and greater FEV_1 and FVC in a sample of 2,633 adults from Nottingham [92]. A 9 years follow-up in these adults carried out by McKeever *et al.* reported that a higher average intake of vitamin C was related to a 50.8 (95% CI 3.8 to 97.7) smaller decrease of FEV_1 during that period. The authors confirmed the previous cross-sectional observation of a greater FEV_1 associated to a higher intake of vitamin C [93](Table 3.1). These results are in keeping with those of the MORGEN study, a large cross-sectional survey of the prevalence of risk factors for chronic diseases, carried out in 6,555 adults aged 20 to 59 years from The Netherlands. The intake of vitamin C was in average well above recommended values in this population, and was associated with a greater FEV_1 and FVC when comparing the 90^{th} versus the 10^{th} percentile of intake [94].

Chen *et al.* assessed the relation between waist circumference, lung function and dietary intake of antioxidants in 1,804 men and women separately [95]. They reported a statistically significant greater FEV_1 in men but not in women with increasing intake of vitamin C, the effect size being half in women compared to that of men in the single linear regression (Table 3.1). In both groups, lung function showed a statistically significant negative association with waist circumference, possibly explained on the grounds that a large waist circumference could affect the movement of the diaphragm and the chest wall. In the multivariable model, where other antioxidants and waist circumference were added as potential confounders, the association between FEV_1 and vitamin C only remained in men. The authors hypothesised that the fact that energy was a strong predictor of lung function in women but no in men, and the higher vitamin C observed in women, could have partly explained the lack of association found in women.

Another cross-sectional study that included a sample of 1,616 Caucasian and Afro American adults, reported than FEV_1% and FVC values were greater with higher quartiles of vitamin C intake. The multivariable analyses showed that vitamin C was positively associated with both FEV_1% and FVC when added separately, but not simultaneously with vitamin E and several carotenoids [91]. Similarly, Hu and colleagues [96] reported that adults from the Third National Health and Nutrition Examination Survey (NHANES) had a small but not statistically significant increase in

FEV_1 as the intake of vitamin C increased, which disappeared when this antioxidant was simultaneously included with vitamin E and carotene in the multivariable model (Table 3.1).

Table 3.1: Association between dietary intake of vitamin C and lung function in adults in cross- sectional studies

First Author	Intake analysed	Outcome measurement of lung function	Difference observed in ml (95% CI) except*	Adjustment
Britton J [92]	1SD increase in intake	FEV_1 FVC	25 (5.2 to 44.8) 23.3 (0.9 to 45.7)	Age, sex, height, mean allergen skin wheal diameter, and pack-years smoking history
Grievink L [94]	90^{th} vs. 10^{th} percentile of intake	FEV_1 FVC	52.9 (23.0 to 82.3) 79.0 (42.3 to 115.7)	Age, age^2, sex, smoking status, pack years of smoking, and TEI
McKeever TM [93]	100 mg /day intake increase	Cross-sectional study: FEV_1	66.8 (12.2 to 121.4)	Age, age^2, height sex, BMI, atopy, TEI, smoking and social class
Chen R [95]	100 mg intake increase	Men FEV_1 FVC Women FEV_1 FVC	10.2 (0.8 to 19.6) 7.4 (-4.0 to 18.0) -1.9 (-4.0 to 7.8) -0.4 (-6.0 to 7.0)	Age, height, weight, working status, TEI, smoking pack years, and serum cotinine, waist circumference, and vitamin E, retinol and β–carotene.
Schunemann HJ [91]	1 SD increase in intake	FEV_1%* FVC%* FEV_1%* FVC%*	1.04 (0.18 to 1.90) 0.85 (0.03 to 1.66) 0.40 (-0.62 to 1.41) -0.24 (-1.21 to 0.72)	Smoking status, total pack-years of smoking, weight, education, eosinophil count, and TEI All above + vitamin E and carotenoids
Hu G [96]	1 SD increase in intake	FEV_1	9.5 (-0.2 to 19.2) -3.0 (-15.5 to 9.4)	Age, age^2, sex, height, race, BMI, income, smoking, and TEI (vitamin C as only nutrient in the model) All above + vitamin E and carotene
Tabak C [97]	Above vs. below median intake	FEV_1: Finland Italy The Netherlands	 5.0 (-76.0 to 85.0) 10.0 (-61.0 to 81.0) 7.0 (-81.0 to 94.0)	Age, height, smoking, BMI, alcohol consumption, and TEI
Butland B [100]	1 SD increase in intake	FEV_1	26.2 (-2.0 to 54.4)	Age, height, age^2, height^2, BMI, smoking history, social class, work exercise and leisure exercise, and TEI
Hu G [98]	100 mg intake increase	FEV_1 FVC	21.6 (-0.4 to 43.5) 24.9 (0.2 to 49.6)	Sex, age, height, weight, TEI, tobacco smoking, and education
Dow L [99]	100 mg intake increase	FEV_1 FVC	0.07 (-0.1 to 0.3) 0.1 (-0.01 to 0.3)	Age, sex, height, current/ex-smoker, and TEI

The evidence on the possible benefit of dietary vitamin C on lung function is contentious, as the findings of greater FEV_1 or FVC related to a higher intake of vitamin C have not been confirmed in at least four large cross-sectional studies. These describe different levels of intake, and different adjustments for potential confounders, showing some, but statistically and biologically weak indication of a greater lung function with higher intakes of vitamin C. Tabak and colleagues found no statistically significant increase in FEV_1 in men from Finland, Italy and The Netherlands when comparing those above versus below the median intake, after controlling for several potential confounders including BMI, smoking, alcohol and energy intake [97]. Butland *et al.* found that FEV_1 was positively associated with vitamin C in a sample of 2,136 men aged 45 to 59 years old before, but not after controlling for social class, and TEI. Adults from rural China have shown an association between FVC but not FEV_1 and dietary intake of vitamin C [98], while in individuals aged 65 or over it has been observed a very small size effect in the intake of this vitamin and measurements of lung function [99] (Table 3.1).

Most of the available evidence on the relation between serum and plasma levels of vitamin C with lung function is suggestive of a positive association (Table 3.2). Two large cross-sectional studies, one from Scottish adults [101] and the other on American adults from the NHANES III survey [96], have reported a statistically significant positive association between FEV_1 and plasma [101] and serum [101] levels of vitamin C. In the NHANES III the authors found that after controlling for vitamins E and A, and selenium in the multivariable analyses, the effect size was smaller but still statistically significant.

Schunemann *et al.* found that a 1 SD increase in vitamin C was positively and statistically significantly related to FEV_1%. They also reported some increase in FVC% but this did not reach statistical significance [102]. Ness and colleagues found that higher plasma levels of vitamin C were associated with a greater FEV_1 in men but not in women selected from the European Prospective Investigation into Cancer (EPIC) [103] (Table 3.2).

Table 3.2: Association between blood levels of vitamin C and lung function in cross-sectional studies

First author	Blood level compared	Outcome measurement of lung function	Difference observed in ml (95% CI) except *	Adjustment
Kelly Y [101]	Increase of 1 SD (as Z score) in plasma levels	FEV_1	49.0 (13.0 to 85.0)	Age, height, age^2, height^2, BMI, sex, smoking, social class, activity level, and other plasma analytes (vitamins A, E and β–carotene)
Hu G [96]	Increase of 1 SD in serum level	FEV_1	28.1 (18.7 to 37.6) 16.6 (7.3 to 26.0)	Height, sex, age, race, BMI, income, and smoking (vitamin C as only nutrient in the model) All above + vitamins E and A, and selenium included in model simultaneously
Schunemann HJ [102]	1 SD increase in serum levels	FEV_1%	0.77 (0.01 to 1.53)*	Weight, blood eosinophils count, education, smoking status, and cumulative tobacco smoke exposure
		FVC%	0.49 (-0.24 to 1.22)*	
Ness AR [103]	Increase in 50μmol/L	Men		Age, height and packet years of cigarette smoking
		FEV_1	22.0 (10.0 to 33.0)	
		FVC	23.0 (9.0 to 37.0)	
		Women		
		FEV_1	4.0 (-2.0 to 10.0)	
		FVC	6.0 (-1.0 to 13.0)	

* Results expressed as regression coefficient of the FEV_1 and FVC as percentages of predicted value (95% CI)

3.2.2.2 Vitamin C and Respiratory Symptoms

Evidence that vitamin C could be related to asthma was initially reported by Puglisi in the 1970s after a experimental model in guinea pigs demonstrated that insufficient intake of a form of vitamin C, L-ascorbic acid, led to a higher contraction of the trachea explained by a reduction of PGE_2 and increased formation of $PGF_{2\alpha}$ [104]. One of the earliest clinical studies examining the relation between vitamin C and asthma was conducted by Olusi *et al.*, who reported that adults with untreated and salbutamol-

treated asthma had significantly lower levels of plasma concentration of vitamin C when compared to controls [105].

Table 3.3 summarizes the current evidence of association between asthma, BHR and atopy, with dietary intake of vitamin C. These results come largely from cross-sectional [94, 106-108], case-control studies [109-113] and one longitudinal study [114].

Grievink and colleagues assessed the association between several respiratory outcomes and intake of vitamin C in Dutch adults. They found that cough was the symptom for which a statistically significant association was reported in those in the 90th percentile of vitamin C intake compared to those in the 10th percentile. For the other four symptoms there was a lack of association [94].

A study on adult Norwegians found that those in the highest tertile of intake per week had a lower prevalence of morning cough, but such intake was unrelated to other four respiratory symptoms, including chest tightness and asthma attack when the whole population was included in the multivariable analyses (Table 3.3). When stratifying by smoking (current/ex/never smoker), current smokers tended to have a lower prevalence of morning cough and chronic cough, while ex smoker in the highest tertile of vitamin C intake had a lower prevalence of wheeze (OR 0.56; 95% CI 0.35-0.88) [107].

Other two cross-sectional studies and one longitudinal survey have found a lack of association between prevalence of asthma, BHR, and atopy [106], wheezing in the last 12 months [108], and incidence of asthma [114]. Schwartz and colleagues examined the relation between several respiratory symptoms and dietary intake of vitamin C, bronchitis being the only symptom negatively associated with vitamin C after controlling for potential confounders [108]. Troisi *et al.* reported a statistically significant negative association between incidence of asthma in women only when intake of vitamin C included consumption of supplements [114] (Table 3.3).

Table 3.3: Association between dietary intake of vitamin C and symptoms of asthma, BHR or atopy in adults

First author	Type of study	Dietary intake compared	Respiratory outcome	OR (95% confidence interval) [Adjusted for], except*
Grievink L [94]	Cross-sectional 6,555 adults The Netherlands	90^{th} vs. 10^{th} percentile	-Cough -Phlegm -Productive cough -Wheeze -Shortness of breath	0.66 (0.50 to 0.87) 0.77 (0.59 to 1.02) 1.09 (0.93 to 1.28) 1.04 (0.83 to 1.30) 0.81 (0.61 to 1.07) [Age, sex, smoking status, pack years of smoking, and energy intake]
Omenaas E [107]	Cross-sectional 3,450 adults Norway	Highest vs. lowest tertile of intake (mg/week)	-Morning cough -Chronic cough -Wheeze in the last 12 months -Breathless at night -Chest tightness -Asthma attack	0.70 (0.55 to 0.90) 0.76 (0.52 to 1.01) 0.86 (0.67 to 1.12) 0.93 (0.59 to 1.51) 1.13 (0.81 to 1.56) 1.47 (0.76 to 2.77) [Age, sex, BMI, occupational exposure, and smoking habits]
Woods RK [106]	Cross-sectional 1,601 adults Australia	Increase per mg/day	-Current asthma [&] -Asthma -BHR -Atopy	0.93 (0.65 to 1.32) 1.19 (0.88 to 1.60) 0.96 (0.71 to 1.31) 0.97 (0.74 to 1.27) [Age, sex, smoking, BMI, region of birth, family history of asthma, and TEI]
Schwartz J [108]	Cross-sectional 9,074 adults USA	2 SD change in intake	-Bronchitis	0.70 (0.54 to 0.92) [Age, sex, race, pack-years of cigarette smoking, family income, and TEI]
Troisi RJ [114]	Prospective cohort (Over a 10-yr period) 77,866 women USA	Highest vs. lowest quintile	-Incidence of asthma (RR including vitamin C supplements) -Incidence of asthma (RR excluding vitamin C supplements)	1.69 (1.28 to 2.23) [Age, smoking, BMI, area of residence, quintiles of energy intake, vitamin C and E simultaneously (including supplements)] 1.11 (0.69 to 1.77) [Age, smoking, BMI, area of residence, quintiles of energy intake, vitamin C and E simultaneously (excluding supplements)]

[&]Current asthma defined as having wheeze in the last 12 months and BHR. "Asthma" defined as answering 'yes' to the questions 'Have you had an attack of asthma in the last 12 months?, 'Have you been woken by an attack of shortness of breath any time in the last12 months?', or 'Are you currently taking any medicine for asthma?.

Continuation Table 3.3

First author	Type of study	Dietary intake compared	Respiratory outcome	OR (95% confidence interval) [Adjusted for] except*
Bodner C [109]	Nested case-control (30-years follow up) 94 cases/ 203 controls Scotland	Lowest vs. highest tertile	-Wheezing	1.45 (0.71 to 2.97) [Smoking habit, atopy, family history, social class, sex, and TEI]
Shaheen S [110]	Case-control 607 cases/864 controls England	Highest vs. lowest quintile	-Asthma (Defined according to questions from ECRHS)	0.92 (0.64 to 1.34) [Age, sex, BMI, social class, housing tenure, employment status, whether a single parent, smoking, passive smoke exposure at home, and TEI]
Soutar A [111]	Case-control 51 cases/38 controls Reactors/Non reactors to BHR=29/58 Scotland	Lowest vs. highest tertile	-History of seasonal symptoms (Included allergy, atopy, eczema, diagnose of asthma) -Positive response to BHR	0.95 (0.32 to 2.77) [Age, sex, and smoking] 7.13 (1.91 to 26.71) [Age, sex, and smoking]
Picado C [112]	Case-control 118 cases/121 controls Spain	*Mean intake in those with severity 4 compared to 1	-Severity of asthma (Established according to GINA) 1= intermittent 4 = severe	152.0 (74.0) vs. 177.0 (76.0); (p>0.05) [Energy intake, sex, and age]
De Luis [113]	Case-control 54 cases /54 controls Spain	*Mean intake (SD) in cases vs. controls	-Allergenic asthma (regular follow up patients)	93.1 (63.9) vs.124.0 (70.0); p<0.05 [No adjustments]

Case-control studies have shown no association when dietary intake of vitamin C in cases with wheezing [109], asthma [110], seasonal symptoms [111], severity of asthma [112], and allergenic asthma [113] has been compared to healthy controls. In a sub-sample, Soutar and colleagues reported than those cases with positive response to BHR were more likely to have a low intake of vitamin C [111] (Table 3.3).

As presented in Table 3.4, at present there are a number of cross-sectional and case-control studies that have examined the relation between vitamin C in blood and respiratory symptoms. A large cross-sectional American study reported that those having a 2SD higher plasma concentration of vitamin C were at lower risk of having

wheeze and bronchitis [108]. Contrary, Kelly and colleagues found no association between several respiratory symptoms and plasma vitamin C when adults were asked abut wheezing attacks with shortness of breath and another two respiratory symptoms [101].

Table 3.4: Association between blood levels of vitamin C and asthma symptoms or atopy in adults

First author	Type of study	Level of vitamin C compared	Respiratory outcome	Mean differences, except* [Adjustment]
Schwartz J [108]	Cross-sectional 9,017 adults USA	2 SD change in serum level *OR (95% CI)	-Bronchitis -Wheezing	0.65 (0.48 to 0.88) 0.56 (0.46 to 0.67) [Age, sex, race, pack-years of cigarette smoking, family income]
Kelly Y [101]	Cross-sectional 7,932 adults Scotland	1 SD (Z score) change in plasma level *OR (95% CI)	-Phlegm in the morning in winter -Phlegm ≥ 3 months/year -Wheezing attacks with shortness of breath	1.02 (0.84 to 1.25) 0.94 (0.74 to 1.20) 1.02 (0.84 to 1.24) [Adjusted by age, sex, social class, smoking, activity level, and other plasma analytes (vitamin E and A)]
Ford ES [115]	Cross-sectional 16,541 adults USA	Mean (SD) serum level in each group	-Current, former, and never asthma -Mild, moderate, severe asthma	34.0 (1.32), 32.9 (2.6), and 37.3 (0.82); p>0.05 [No adjustments] 41.1 (1.77), 41.2 (3.1), and 39.6 (3.1); p>0.05 [Age, sex, race, education, smoking status, cotinine, non-high-density lipoprotein, HDL, BMI, physical activity, alcohol consumption]
Mainous A [116]	Cross-sectional 19,760 adults USA	Mean (SD) serum level of vitamin C	Number of ambulatory visits for wheezing in last 12 months	0 42.6± 25.0 µM /L 1 43.2± 28.4µM /L >1 42.6± 27.8 µM /L (p= 0.95)
McKeever TM [117]	Cross-sectional 5,858 adults USA	1 SD difference in serum level	Skin allergen sensitization	0.98 (0.92 to 1.04) [Age, sex, smoking status, BMI, poverty, race/ethnicity, total cholesterol, and tryglicerides]
Vural H [118]	Case-control 40 cases/43 healthy controls	Mean (SD) serum level (µM/L) in each group	-Bronchial asthma	36.9 (12.5) vs. 53.4 (13.1) (p<0.001)

Three groups investigated prevalence of asthma [115], number of ambulatory visits to healthcare units as consequence of episodes of wheeze in the last 12 months [116], and allergen sensitisation [117], and their association with serum levels of vitamin C in the participants of the NHANES III. They found no evidence for an association with any of the outcomes studied (Table 3.4).

Vural *et al.* reported that individuals with asthma had significantly lower serum levels of vitamin C when compared to healthy controls [118].

Overall, this epidemiological evidence suggests that vitamin C may be related to a greater lung function as gathered from studies on its blood level but the evidence from dietary intake is suggestive of a lack of association. Regarding symptoms of asthma, the results from case-control studies and some cross-sectional studies suggest that there is no association between intake of this vitamin with several symptoms of asthma, while the studies on blood levels of vitamin C report mixing results to strengthen a more definitive role of the vitamin in asthma.

3.2.2.3 Vitamin E and Lung Function

Table 3.5 summarises the differences in FEV_1 and FVC according to vitamin E intake in adults. A large cross-sectional study based on a cohort of men aged 50-64 years (n=2,519) from Finland, Italy and The Netherlands, assessed dietary intake of antioxidant vitamins C, E and A and fruits and vegetables. They reported a positive significant association between intake of vitamin E and a greater FEV_1 in men from Finland when controlling for age, height, smoking, BMI, and alcohol consumption. After subsequent adjustment for energy intake, FEV_1 was not associated with intake of vitamin E in any of the countries [97]. The MORGEN study found that intake of vitamin E in this population was not associated with FEV_1 or FVC (Table 3.5) [94].

A cross-sectional analysis by Butland *et al.* of the association between intake of antioxidants and FEV_1 in 2,136 Welsh men aged 45-59 years old that were part of a cohort study showed that intake of vitamin E was associated with maximum FEV_1 in this population. However, such association was present in models that included as confounders BMI, age^2, $height^2$, age, but lost statistical significance when controlled for social class and smoking. When energy intake was subsequently added as

confounder, the size of the association was restored. These authors also added as confounders vitamin C and apple intake, which tended to decrease the size of the association slightly but was still significant [100] (Table 3.5).

Another study in a population of 2,633 adults from UK found that an increase in 1 SD of vitamin E intake was related to a 20.1ml increase in FEV_1 after adjustment for several confounders including smoking but not socio-economic level. In this study, after controlling for vitamin C intake, the association between vitamin E and FEV_1 or FVC was no longer observed [92]. These findings are in agreement with those of the NHANES study, a large cross-sectional survey, which found that dietary intake of vitamin E was associated with a greater FEV_1 in these adults after adjusting for several confounders. However, such association was no longer observed when vitamin C was also included in the multivariable analysis [96] (Table 3.5)

Other two studies have reported a greater lung function in adults with higher intake of vitamin E. A cross-sectional study on 1,616 individuals aged 59.6±10.8 years examining lung function and intake of specific antioxidants found a statistically significant difference in the FEV_1% and FVC% in those with the highest quartile intake of vitamin E compared to those with the lowest [102]. A study in a sample of 188 adults found an independent association between intake of vitamin E and FEV_1 and FVC [99] (Table 3.5).

Studies assessing association between antioxidants in blood and lung function provide are shown in Table 3.6. A non-statistically significant increase in FEV_1 in plasma of 367 Dutch men and women was reported by Grievink *et al.* [119]. More recently, in another population, Grievink *et al.* found a greater FEV_1 in 528 elderly adults with a higher serum level of α–tocopherol but did not reach statistical significance [120]. Hu *et al.* reported that increasing serum levels of vitamin E were statistically significantly associated with a greater FEV_1 in the NHANES III study [96]. These results are in keeping with those reported by Schunemann *et al.* who found a positive association between serum levels of vitamin E and FEV_1% and FVC% in a sample of 1,616 adults [102].

Recently, a study on a cohort of workers and residents highly exposed to asbestos in Australia looking at serum antioxidants and lung function has been published [121].

The study included an average of 5.4 simultaneous (same day) measures of serum antioxidants and spirometry over a period of 6 years. The authors found that serum levels of vitamin E were positively associated with a greater FEV_1 and FVC at the entry of the study, but in contrast, it was observed that the increase in serum vitamin E was related to an annual decline in lung function in this population.

Table 3.5: Association between dietary intake of vitamin E and lung function in adults in cross-sectional studies

First author	Intake analysed	Outcome measurement of lung function	Difference observed in ml except * (95% CI)	Adjustment
Tabak C [97]	Above vs. below median intake	FEV_1: Finland Italy The Netherlands	 23 (-67 to 112) -62 (-143 to 18) -92 (-189 to 5)	Age, height, smoking, BMI, alcohol consumption, and energy intake
Grievink L [94]	90th percentile of consumption vs. 10th	FEV_1 FVC	27.9 (-12.9 to 68.7) 18.2 (-32.2 to 68.6)	Age, age^2, sex, energy intake, smoking status, and pack years of smoking
Butland B [100]	Increase per SD	FEV_1 FEV_1 FEV_1	19.7 (-7.8 to 47.2) 39.1 (9.4 to 68.8) 31.7 (0.9 to 62.5)	Age, height, age^2, height^2, BMI, smoking, and social class All above + energy intake All above + vitamin C and apple
Britton J [92]	Increase per SD	FEV_1 FVC	20.1 (1.3 to 40.4) 23.1 (1.0 to 45.0)	Age, sex, height, mean allergen skin wheal diameter, and pack-years smoking history
Dow L [99]	100 mg intake increase	FEV_1 FVC	4.2 (1.0 to 7.4) 5.3 (1.8 to 8.8)	Age, sex, height, current/ex-smoker, energy intake
Schunemann HJ [91]	Highest vs. lowest quartile	FEV_1% FVC%	4.0 (1.0 to 6.9)* 3.4 (0.6 to 6.2)*	Smoking status, total pack-years of smoking, weight, education, eosinophil count, total daily energy intake
Hu G [96]	Increase per SD	FEV_1 - Nutrient alone in the model - Nutrient included simultaneously with vitamin C and carotene	 16.4 (5.5 to 27.4) 11.5 (-1.4 to 24.5)	 Height, sex, age, age^2, race BMI, income, smoking, TEI, total fat intake

* Results expressed as difference in FEV_1% and FVC% of predicted values

Table 3.6: Association between blood levels of vitamin E and lung function in adults in cross-sectional studies

First author	Blood level analysed	Outcome measurement of lung function	Difference observed in ml except * (95% CI)	Adjustment
Grievink L [119]	90th percentile vs. 10th in plasma	FEV_1 FVC	23.2 (-91.3 to 137.7) 35.6 (-111.2 to 182.4)	Age, sex, height, smoking, alcohol consumption
Grievink L [120]	5th vs. 1st quintile in serum	FEV_1	94 (-63 to 251)	Age, height, sex, pack-years of smoking
Hu G [96]	Increase in 1 SD in serum	FEV_1	49.1 (37.4 to 60.7)	Age, height, sex, race, BMI, income, smoking, serum triglycerides and total cholesterol
Schunemann HJ [102]	Increase in 1 SD in serum	FEV_1%	1.12 (0.34 to 1.90) *	Weight, blood eosinophils count, education, smoking status, cumulative tobacco smoke exposure.
		FVC%	1.66 (0.91 to 2.41) *	
Alfonso S [121]	Highest vs. lowest quartile in plasma	Levels at entry of study FEV_1 FVC Annual change (ml/year) FEV_1 FVC	35.9 (8.1 to 63.7) 82.3 (47.1 to 117.5) -7.9 (-4.3 to -1.6) -13.9 (-21.9 to -6.0)	Age, sex, height, asbestos exposure and smoking history

* Results expressed as regression coefficient (95% CI)

3.2.2.4 Vitamin E and respiratory symptoms, BHR and atopy

Several studies have explored the association between vitamin E and respiratory symptoms (Table 3.7). Four case-control studies failed to find any beneficial association between dietary intake of this vitamin and wheezing [109], asthma [110], seasonal symptoms [111], and severity of asthma [112]. Similarly, a large cross-sectional study in 6,555 Dutch adults found no association between the highest percentile of intake of vitamin E and any of five respiratory symptoms [94].

Table 3.7: Association between dietary intake of vitamin E and asthma symptoms, atopy or BHR in adults

First author	Type of study	Level of vitamin E compared	Respiratory outcome	OR (95% CI) [Adjusted for], except*
Grievink L [94]	Cross-sectional 6,555 adults The Netherlands	90th percentile vs. 10th	-Cough -Phlegm -Productive cough -Wheeze -Shortness of breath	0.85 (0.61 to 1.18) 1.06 (0.76 to 1.47) 1.26 (1.02 to 1.56) 1.13 (0.85 to 1.52) 1.24 (0.87 to 1.77) [Age, sex, TEI, smoking status, and pack years of smoking]
Woods RK [106]	Cross-sectional 1,601 adults Australia	Increase per mg	-Current asthma -Asthma -BHR -Atopy	0.89 (0.43 to 1.85) 0.62 (0.33 to 1.15) 1.26 (0.67 to 2.38) 1.01 (0.58 to 1.77) [Age, sex, smoking, BMI, region of birth, family history of asthma, and TEI]
Fogarty A [122]	Cross-sectional 2,633 adults England	Increase per mg	-Atopy	0.95 (0.92 to 0.99) [Age, sex, and smoking]
Troisi RJ [114]	Prospective cohort 77,866 women USA	Highest vs. lowest quintile of intake	-Incidence of asthma (RR including use of vitamin E supplements) -Incidence of asthma (RR including only vitamin E from diet)	0.83 (0.64 to 1.08) [Age, smoking, BMI, area of residence, quintiles of energy intake, vitamin C and E simultaneously (including supplements)] 0.53 (0.33 to 0.86) [Age, smoking, BMI, area of residence, quintiles of TEI, vitamin C and E simultaneously (excluding supplements)]
Bodner C [109]	Nested case-control (30-years follow up) 94 cases/ 203 controls Scotland	Low vs. highest tertile	-Wheezing	1.93 (0.79 to 4.72) [Smoking habit, atopy, family history, social class, sex, and TEI]
Shaheen S [110]	Case-control 607 cases/864 controls England	Highest vs. lowest quintile	-Asthma (Using questions from ECRHS)	0.76 (0.48 to 1.20) [Age, sex, BMI, social class, housing tenure, employment status, whether a single parent, smoking, passive smoke exposure at home, and TEI]
Soutar A [111]	Case-control 51 cases/38 controls Reactors/non-reactors to BHR=29/58 Scotland	Low vs. high tertile	-History of seasonal symptoms (Included allergy, atopy, eczema, diagnose of asthma) -Reactors to BHR vs. non-reactor	1.50 (0.51 to 4.41) [Age, sex, and smoking] 1.89 (0.59 to 6.11) [Age, sex, and smoking]
Picado C [112]	Case-control 118 cases/121 controls Spain	*Mean intake (SD) in those with severity 4 compared to 1	-Severity of asthma (Established according to GINA) 1= intermittent 4 = severe	6.4 (2.1) vs. 7.6 (1.8); p>0.05 [Age, sex, and TEI]
De Luis [113]	Case-control 54 cases /54 controls Spain	*Mean intake (SD) in cases vs. controls	-Allergenic asthma (regular follow up patients)	5.1 (2.3) vs. 7.3 (1.2) ; p<0.05 [No adjustments]

Woods *et al.* reported lack of association between higher levels of vitamin E intake with current asthma, asthma ever, and BHR in a cross-sectional study [106]. A large prospective cohort study in women reported that a higher intake of vitamin E (excluding or including supplement intake) was associated with a lower risk of incidence of asthma over a period of 10 years [114] (Table 3.7).

In relation to atopy, two cross-sectional surveys have reported different findings. Woods *et al.* found no association between atopy and increase per mg of dietary intake of vitamin E in a sample of 1,601 Australian adults, with a prevalence of atopy of 62.8% defined as a ≥ 3mm wheal diameter in response to any out of eight allergens [106]. Contrary, Fogarty *et al.* reported a reduction in the risk of being atopic as daily intake of vitamin E increased in a sample of 2,633 adults from Nottingham. They defined atopy as a ≥ 1mm wheal diameter in response to any of three allergens. This study also found that intake of vitamin E was independently associated with a reduction in the serum levels of IgE, and they suggested that atopic responses mediated by IgE might be reduced by the effect of vitamin E [122].

Evidence of association between asthma symptoms and blood levels of vitamin E is presented in Table 3.8. A large cross-sectional study on Scottish individuals aged 16 to 64 years found a negative association of statistical significance between a 1 SD change in plasma vitamin E and phlegm in morning in winter in the multivariable analysis, but found no association with the other two respiratory symptoms studied [101] (Table 3.8).

Another two cross-sectional studies on adults recruited from the Third National Health and Nutrition Examination Survey (NHANES III) and studied serum levels of vitamin E in relation to atopy [117] and asthma prevalence and severity [115]. In the first, McKeever *et al.* found that serum vitamin E was statistically significantly associated with positive sensitisation in a sample of over 5,000 adults of whom 57.6% had a positive response to any of ten skin prick tested allergens. However, they did not find it in a sample of over 4,000 children analysed in parallel, with a similar prevalence of atopy [117]. The study by Ford *et al.* assessed whether serum vitamin E in adults was related to self-reported current doctor-diagnosed asthma or former asthma (answering no to the question 'Do you still have asthma'?), as well as to severity. Vitamin E levels

were similar in all the groups compared (Table 3.8) and were not related to prevalence of asthma or its severity in the multiple linear regressions (data not shown in the paper) [115].

Table 3.8: Association between blood levels of vitamin E and asthma symptoms or atopy in adults

First author	Type of study	Level of vitamin E compared	Respiratory outcome	OR (95% CI) [adjustment] except*
Kelly Y [101]	Cross-sectional 7,932 adults Scotland	1 SD score (Z score) change in plasma concentration	- Phlegm in the morning in winter	0.80 (0.65 to 0.99)
			- Wheezing attacks with shortness of breath	0.93 (0.77 to 1.13)
			- Phlegm ≥ 3 months per year	0.87 (0.68 to 1.11) [All three analyses adjusted for age, social class of head of household, smoking status, activity level, and vitamin A and C)
McKeever TM [117]	Cross-sectional 5,858 adults USA	1 SD difference in serum level	Skin allergen sensitization	0.93 (0.87 to 0.99) [Age, sex, smoking status, BMI, poverty, race/ethnicity, total cholesterol, and tryglicerides]
Ford [115]	Cross-sectional 16,541 adults USA	*Mean (SD) of serum level in each group	-Current, former, and never asthma	24.3 (0.38), 27.3 (1.72), and 24.4 (0.19); p>0.05 [unadjusted]
			-Mild, moderate, severe asthma	28.2 (0.95), 27.3 (1.23), 25.9 (1.11); p>0.05 [Age, sex, race, education, smoking status, cotinine, non-high-density lipoprotein, HDL, BMI, physical activity, alcohol consumption]
Bodner C [109]	Nested case-control (30-years follow up) 86 cases/ 194 controls Scotland	1 unit increase in plasma concentration	Current wheezing	1.03 (0.99 to 1.07) [Age, sex, smoking habit, atopy, family history, and social class]
Gazdik F [123]	Case-control 56 cases/25 controls Slovak Republic	*Median (IQR) plasma concentration in cases vs. controls (µm/L)	- Mild to moderate persistent asthma as defined by GINA vs. healthy controls.	24.1 (19.8 to 30.5) vs.33.2 (28.25 to 38.05) p=0.006

Evidence from a case-control study in Scotland found that plasma concentration of vitamin E was unrelated to current wheezing. They found no association between vitamin E and prevalence of current wheezing when analyses included all 86 cases (those having responded yes to the question 'Have you had an attack of wheezing after the age of 15 years?', or those with current wheezing were considered as cases (n=59) [109]. A smaller clinical case-control study assessing levels of the antioxidant coenzyme Q_{10} in asthmatics, also measured a number of antioxidant nutrients, and reported no differences between levels of vitamin E in plasma in those with mild to moderate persistent asthma versus healthy controls [123].

Overall, the evidence for an association between vitamin E seems to suggest some consistent beneficial association between lung function and dietary vitamin E. Such an association did not occur between lung function and blood vitamin E level. There is lack of supportive evidence for an association between dietary intake or blood levels of vitamin E and asthma. Evidence for an association between atopy and dietary intake of blood levels of vitamin E is still limited and inconsistent.

3.2.2.5 Vitamin A and lung function

Intake of β-carotene was positively associated with better lung function in several studies. In a cohort study on men from three countries, Tabak *et al.* found a positive association between intake of β–carotene and FEV_1 in those from The Netherlands, but not in the men from Italy and Finland [97]. In agreement with these findings, a positive association was found in a sample of Dutch adults aged 20-59 [94]. Butland *et al.* also reported a positive association but it disappeared after controlling for smoking history in Welshmen of a similar range of age [100] (Table 3.9).

Observational studies assessing the association between blood levels of carotenoids and lung function offer further support to a possible protective effect of these antioxidants in respiratory health. Two cross-sectional studies in Dutch adult [119] and elderly population [120] reported that plasma and serum levels of several carotenoids were related to a greater lung function (Table 3.10). This positive association was also

confirmed by Hu and colleagues in adults from the NHANES III survey [96], but not by others [101].

Table 3.9: Association between dietary intake of vitamin A and its precursors and lung function in adults

First Author	Intake analysed	Outcome measurement of lung function	Difference observed in ml (95% CI) except*	Adjustment
Tabak C [97]	Above vs. below median intake β–carotene	FEV_1: Finland Italy The Netherlands	 -15.8 (-35.5 to 3.9) 1.1 (-55.6 to 57.9) 141.1 (27.4 to 254.7)	Age, height, smoking, BMI, alcohol consumption, and TEI
Grievink L [94]	90^{th} vs. 10^{th} percentile of intake β–carotene	FEV_1 FVC	60.0 (31.4 to 88.6) 75.2 (40.2 to 110.2)	Age, age^2, sex, smoking status, pack years of smoking, and TEI
Butland B [100]	1 SD increase in intake β–carotene	FEV_1	14.1 (-13.9 to 42.1)	Age, height, age^2, $height^2$, BMI, smoking history, social class, work exercise and leisure exercise, and TEI
Hu G [96]	1 SD increase in intake β–carotene	FEV_1	18.2 (8.7 to 27.6) 16.1 (4.2 to 28.1)	Age, age^2, sex, height, race, BMI, income, smoking, and TEI (vitamin C as only nutrient in the model) All above + vitamin E and carotene

A cross-sectional study on American adults looked at the association between blood levels of six different carotenoids (including β–carotene) and FEV_1% and FVC%, finding a positive association only with lutein, a carotenoid with lower antioxidant capacity than β–carotene. An increase in both spirometric measurements was observed as the intake lutein increased (difference between highest vs. lowest quartile 2.8%, p=0.016 and 4.4%, p<0.001, respectively) [91]. Finally, Chewers and colleagues reported that higher serum levels of β–carotene and to a lesser extent retinol, were positively related to a greater lung function in a population of adults exposed to high levels of asbestos [124] (Table 3.10).

Table 3.10: Association between lung function and blood levels of vitamin A (retinol) or its precursors

First author	Blood level compared	Outcome of lung function	Main results		Adjustment
Grievink L [119]	Plasma level difference (µmol/L) between the 90th vs. the 10th percentile of β-carotene	FEV_1 FVC	b= 73.0 (59.9) * b=147.4 (75.6)		Age, height, sex, pack-years of smoking, and alcohol consumption
Grievink L [120]	Highest vs. lowest serum quintile (µmol/L): -Total carotenoids -α-carotene -β-carotene -Lycopene	FEV_1	Difference in FEV_1: 210 (56 to 365) (p=0.004) 257 (99 to 414) (p=0.02) 195 (40 to 351) (p=0.01) 171 (16 to 325) (p=0.004)		Age, height, sex, and pack-years of smoking
Hu G [96]	1 SD increase in serum level (µg/dL) of β-carotene	FEV_1	Difference in FEV_1: 27.5 (16.1 to 39.0)		Height, sex, age, race, BMI, income, smoking, serum triglycerides and total cholesterol
Kelly Y [101]	1 SD(µmol/L) change in plasma level of: Vitamin A β-carotene	FEV_1	Difference in FEV_1: 22 (-14 to 58) 11 (-23 to 45)		Age, social class, smoking status, activity level and other plasma analytes
Schunemann HJ [102]	1 SD (µg/mL) serum level change: -β-cryptoxanthin -Lutein/zeaxanthin -β-carotene -Lycopene -Retinol	FEV_1% FVC%	FEV_1% * b=1.52 (0.41) b=0.90 (0.39) b=0.67 (0.40) b=0.21 (0.39) b=0.92 (0.39)	FVC% * b=1.63 (0.39) b=1.38 (0.38) b=1.02 (0.38) b=0.08 (0.37) b=0.66 (0.37)	Weight, blood eosinophil count, education, smoking status.
Chuwers P [124]	75th vs. 25th percentile of serum level (ng/mL): -β-carotene -Retinol	 FEV_1 FVC FEV_1 FVC	 b=0.06 (0.03) (p<0.05)* b=0.09 (0.04) (p<0.05)* b=0.17 (0.09) (p=0.08)* b=0.23 (0.11) (p=0.05)*		Asbestos exposure, and smoking

* Results expressed as regression coefficients (standard error) of multiple linear regression

3.2.2.6 Vitamin A and respiratory symptoms, BHR or atopy

Almost all the data collected to date from observational studies have shown no association between intake of retinol, β-carotene and various respiratory symptoms of asthma, BHR and physician-diagnosed asthma [94, 106, 109, 110, 112, 114] (Table 3.11). Some authors have found a positive association between intake of β-carotene

and wheeze [94], hay fever [125], as well as a higher intake of retinol in asthmatic subjects [113]. Woods et al reported a negative association between retinol intake and asthma [106] (Table 3.11).

Table 3.11: Association between dietary intake of vitamin A and carotenoids and respiratory symptoms of asthma, BHR or atopy in adults

First author	Type of study	Level of vitamin/carotenoid compared	Respiratory outcome	OR (95% CI) [Adjusted for], except*
Grievink L [94]	Cross-sectional 6,555 adults The Netherlands	90^{th} percentile vs. 10^{th} β–carotene (µg/day)	-Cough -Phlegm -Productive cough -Wheeze -Shortness of breath	0.86 (0.67 to 1.10) 1.11 (0.87 to 1.40) 1.14 (0.99 to 1.33) 1.27 (1.04 to 1.55) 1.00 (0.77 to 1.29) [Age, sex, smoking status, pack years of smoking, and TEI]
Woods RK [106]	Cross-sectional 1,601 adults Australia	Increase per mg Retinol (µg/day)	-Current asthma -Asthma -BHR -Atopy	0.77 (0.54 to 1.10) 0.71 (0.53 to 0.96) 0.96 (0.70 to 1.31) 1.01 (0.76 to 1.33) [Age, sex, smoking, BMI, region of birth, family history of asthma, and TEI]
Troisi RJ [114]	Prospective cohort 77,866 women USA	Highest vs. lowest quintile of β–carotene (IU)	-Incidence of asthma	Relative risk: 0.82 (0.65 to 1.05) [Age, smoking, BMI, area of residence, quintiles of TEI, intake of vitamin C and E]
Bodner C [109]	Nested case-control (30-years follow up) 94 cases/ 203 controls Scotland	Low vs. highest tertile β–carotene (µg/day)	-Wheezing	0.88 (0.45 to 1.75) [Smoking habit, atopy, family history, social class, sex, and TEI]
Shaheen S [110]	Case-control 607 cases/864 controls England	Highest vs. lowest quintile β–carotene (µg/day)	-Asthma (Using questions from ECRHS)	1.43 (0.98 to 2.09) [Age, sex, BMI, social class, housing tenure, employment status, whether a single parent, smoking, passive smoke exposure at home, and TEI]
Soutar A [111]	Case-control 51 cases/38 controls Reactors/non-reactors to BHR=29/58 Scotland	Low vs. high tertile Retinol (µg/day) β–carotene (µg/day)	-History of seasonal symptoms (Included allergy, atopy, eczema, diagnose of asthma) -Reactors to BHR vs. non-reactor	Retinol: 0.64 (0.23 to 1.80) β–carotene : 1.53 (0.52 to 4.53) [Age, sex, and smoking] Retinol: 1.13 (0.35 to 3.64) β–carotene : 2.00 (0.63 to 6.33) [Age, sex, and smoking]

Continuation Table 3.11

First author	Type of study	Level of vitamin/carotenoid compared	Respiratory outcome	OR (95% CI) [Adjusted for], except*
Picado C [112]	Case-control 118 cases/121 controls Spain	*Mean intake (SD) in those with severity 4 compared to 1 Retinol (µg/day)	-Severity of asthma (Established according to GINA) 1= intermittent 4 = severe	968.0 (658) vs. 1005.0 (981); p>0.05 [Age, sex, and TEI]
De Luis DA [113]	Case-control 54 cases /54 controls Spain	*Mean intake (SD) of retinol (µg/day) in cases vs. controls	-Allergenic asthma (regular follow up patients)	459 (242) vs. 642 (399) ; p<0.05 [No adjustments]
Nagel G [125]	Case-control 334 cases/1,336 controls Germany	Highest vs. lowest quartile intake of β–carotene (µg/day)	Hay fever	-1.69 (1.69 to 2.63) [Age, sex, educational level, sports activity, smoking status, BMI, TEI]

The evidence from studies assessing blood levels of vitamin A or carotenoids, although limited to few studies, suggests that there is no association between these nutrients and current wheeze [109], skin allergen sensitisation [117], and the serum levels found in asthmatic subjects do not differ from those without asthma [115] (Table 3.12).

Overall, the current evidence is suggestive of some beneficial effect of vitamin A and carotene on lung function, but not on asthma. Further studies are needed to elucidate whether the effect of vitamin A is limited only to lung function.

3.2.3. Randomised controlled trials on vitamins and lung function or asthma symptoms

The issue of whether supplementation with antioxidants may have a beneficial effect on asthma has received growing attention, mainly based on the assumption that a pro-oxidant stage co-exists with this disease. There are a number of clinical trials developed and discussed in the literature, regarding the use and possible advantages of supplementation with antioxidant vitamins, vitamin C being one of the earliest and most studied (Table 3.13). One of the first studies published in 1979 showed no benefits after short-term administration of this vitamin to adults [126]. Another two studies in larger samples of individuals confirmed the lack of effect [127, 128]. These studies suggested that vitamin C has no acute bronchodilator effect and does not alter

BHR in asthmatic subjects. However, two studies published in the 1980s found a favourable effect of long term [129] and double dose-only [130] administration of vitamin C in asthmatic subjects, opening a new debate.

Table 3.12: Association between blood levels of vitamin A and carotenoids and asthma symptoms or atopy in adults

First author	Type of study	Level of vitamin/carotenoid compared	Respiratory outcome	OR (95% CI) [adjustment] except*
Bodner C [109]	Nested case-control (30-years follow up) 86 cases/ 194 controls Scotland	1 unit increase in plasma concentration of -Retinol (µmol/L) -β–carotene(µmol/L)	Current wheezing	1.29 (0.95 to 1.74) 0.35 (0.12 to 1.01) [Age, sex, smoking habit, atopy, family history, and social class]
McKeever TM [117]	Cross-sectional 5,858 adults USA	1 SD difference in serum level of -Retinol (µg/dL) -β–carotene(µg/dL)	Skin allergen sensitization	1.03 (0.97 to 1.09) 0.96 (0.91 to 1.01) [Age, sex, smoking status, BMI, poverty, race/ethnicity, total cholesterol, and tryglicerides]
Ford ES [115]	Cross-sectional 16,541 adults USA	*Mean serum level (µmol/L) -Retinol -β–carotene -Lycopene	-Current asthma vs. never asthma -Mild, moderate or severe asthma	Retinol: 2.01 vs. 2.0 β–carotene:0.31 vs. 0.32 Lycopene: 0.44 vs. 0.44 [unadjusted] Retinol: 2.08, 2.08 and 2.06 β–carotene: 0.40, 0.36 and 0.32 Lycopene: 0.43, 0.44, and 0.40 [Age, sex, race, education, smoking status, cotinine, non-high-density lipoprotein, HDL, BMI, physical activity, alcohol consumption]

The hypothesis of whether a combined dose of antioxidant vitamins could better show a beneficial effect in lung function and asthma outcomes has also been explored (Table 3.14). Two studies carried out in adults suggest that supplementation with the three antioxidant vitamins promotes a well maintained lung function in adults exposed to air pollutants [131] or to intense exercise [132] when compared to those receiving placebo.

Contrary, the largest randomised controlled trial so far carried out, which included 300 asthmatic subjects allocated to three different types of supplementation treatment, reported a lack of association in relation to lung function measurements and BHR [133].

Neuman *et al.* found that subjects supplemented with lycopene [134] or β–carotene [135] for 7 days had a lower reduction of FEV_1 after exercise-induced asthma. Trenga and colleagues studied BHR to sulphur dioxide (SO_2) and lung function measures in a sample of 17 asthmatic subjects supplemented with vitamin E and vitamin C in a double-blind cross-over design, finding that those receiving the supplement responded less severely to SO_2 and had lower changes in their lung function measurements when compared to the placebo group [136].

Table 3.13: Effect of supplementation with vitamin C in asthmatic adults

First author	Study design	Type and No. of participants	Length of trial and dose administered	Outcome	Main results
Anah CO [129]	Randomised, double blind placebo controlled trial	41 individuals Non-smokers Asthmatics for at least 4 years	14 weeks 1 g daily (n=22) placebo pill (n=19)	Asthma attacks	Those under vitamin C suffered lower and less severe attacks during trial ($p<0.01$)
Kordansky DW [126]	Randomised double-blind placebo cross-over design	6 individuals Asymptomatic sensitive asthma (defined by skin-prick test)	7 days 500 mg daily	FEV_1 PD35SGaw (provocation dose necessary for a 35% reduction in specific airways conductance) Tested on day 7	No changes in baseline FEV1 ($p>0.70$) No significant changes in PD35SGaw
Malo JL [127]	Randomised double blind placebo controlled cross-over	16 individuals Asthma	4 separate days 2g of vitamin C	PC_{20} (histamine challenge) assessed four times one hour after administration of vitamin C	No difference between PC_{20} on days 2, 3, and 4 and by standardizing for the four PC_{20} results obtained on day 1
Schachter EN [130]	Randomised double-blind controlled trial, cross-over	12 adults Exercise-induced asthma Never on corticosteroids	Single dose of 500 mg of vitamin C in two subsequent days.	FEV_1, FVC and PEF after and before exercise	FVC 0.23 ± 0.08 L in treated group vs. 0.48± 0.14 L in placebo group. The authors suggest a mild anti-bronchospastic action of vitamin C in EIA subjects.
Cohen HA [128]	Randomised double-blind	20 adults physician-diagnosed asthma	Single dose of 2 g of vitamin C one hour after a 7-minute exercise session on a treadmill	FEV_1 FVC PD_{20} (methacholine challenge)	No changes in lung function measurements 9 patients showed a significant reduction in PD_{20}

Table 3.14: Effect of supplementation of combined antioxidants on lung function and asthma in adults

Antioxidant/ Type study	First author	Population	Dose /length	Outcome	Main results
Lung function					
Vitamin C, E and β–carotene Randomised, double blind placebo, cross over	Romieu I [131]	47 shoe-cleaners (Street workers) exposed to high levels of ozone and NO_2 in air	650 mg vitamin C+ 75 mg vitamin E+ 15 mg β–carotene Two phases (10/8 weeks each)	-FEV_1 -FVC -FEF_{25-75}	Phase 1 and 2: In supplemented group no association between any parameter of lung function and ozone or NO2 exposure. Significant inverse association with most of them (except FVC) in placebo
Vitamin C, E and β–carotene Randomised, controlled trial	Grievink L [132]	Healthy cyclists 12 supplemented 14 control	650 mg vitamin C+ 75 mg vitamin E+ 15 mg β–carotene 3 months	-FEV_1 -FVC -PEF	Significant difference in FVC in supplemented vs. controls. Lung function in supplemented group was no association with ozone exposure
Asthmatic subjects					
Vitamin C & Magnesium (Mg) Randomised, double blind placebo	Fogarty A [133]	300 individuals Physician-diagnosed asthma	1 g/d vit. C + Mg placebo 450 mg/d Mg + vit C placebo Vit C+ Mg placebo 16 weeks	-FEV_1 -FVC -PD_{20}	No significant changes in any of the groups
Lycopene Randomised, double blind placebo	Neuman I [134]	11 supplemented 9 placebo Exercise-induced-asthma	30 mg/d lycopene 7 days/4 weeks washout/7days	FEV_1	Supplemented group had a mean reduction after exercise of -14.7% ΔFEV_1 vs. -26.5% in placebo
β–carotene Randomised, double blind placebo	Neuman I [135]	38 supplemented 20 placebo Exercise-induced-asthma	64 mg/d β-carotene 7 days	FEV_1	Higher reduction in post-exercise FEV_1 in placebo group.
Vitamin C & E Randomised, double blind placebo	Trenga C [136]	17 non-smokers Physician-diagnosed asthma	400 UI Vitamin E + 500 mg Vitamin C 5 weeks (before exposure to SO_2 /exercise challenge)	FEV_1 FVC FEF_{25-75} PEF	Treated vs. placebo: FEV_1 -1.2% vs. 4.4% PEF +2.2% vs. -3.0% FEF_{25-75} +2.0% vs. -4.3%

The studies on supplementation with antioxidant vitamins arise two issues. Firstly, the small sample size of these studies may have had insufficient statistical power to assess the hypothesis. Secondly, it is unclear whether a dose well above the daily requirements may be needed for a long period of time to produce a favourable effect, or

at the contrary, whether once a single high dose is given, it will provide sufficient protection against oxidative imbalance in the individual.

3.2.4. Antioxidant-related minerals and the experimental evidence

A number of minerals participate in the endogenous antioxidant system. Their presence contributes to maintain the antioxidant capacity of the endogenous antioxidant enzymes CAT, GSH-Px and SOD, which play a central role in the antioxidant-oxidant balance. Selenium is an essential trace mineral that is part of the antioxidant enzyme GSH-Px.

In relation to magnesium, there is evidence that deficiency of magnesium is related to oxidative stress, as it increases histamine production and synthesis of NO [137]. Deficiency of magnesium also stimulates the phagocytes, which produce ROS [138]. Although the precise mechanisms of these responses to deficiency of magnesium are not well understood, it has been suggested that deprivation of magnesium may increase cellular vulnerability to oxidation by depleting reduced glutathion (GSH) [139]. Another effect of deficiency of magnesium has been explained by its interaction with calcium, as lower concentrations of the former leads to a higher influx of intra-cellular calcium facilitating bronchial smooth-muscle contraction, thereby being a risk factor for airway obstruction [140].

Zinc is a mineral with at least four antioxidant-related properties: (1) preventing protein oxidation; (2) removing iron and copper from the cell membrane (therefore preventing lipid peroxidation); (3) directly neutralizing ROS by accepting their spare electrons; (4) as a component of the antioxidant enzyme Cu-Zn SOD. In addition to these antioxidant properties it has been postulated that zinc may be related to anti-inflammatory properties, as its deficiency has been related to an exacerbation of eosinophils and neutrophils activation and to a release of interleukins 4 and 5 (IL-4 and IL-5, respectively), leukotrienes B_4 and prostaglandin E_2, components of the inflammatory response of asthma [141].

3.2.5 Minerals and the epidemiological evidence

Most of the evidence currently available from observational studies in relation to lung function or asthma symptoms and minerals with some antioxidant role, has included

selenium and magnesium, with still few studies evaluating the dietary intake of these minerals. Zinc has been mainly studied in relation to symptoms of asthma and BHR, but little is known on its role in lung function in general adult population.

3.2.5.1 Minerals and lung function

a) Selenium

The role of selenium in lung function has been seldom studied in community studies, with some evidence that a higher level of blood level of selenium is related to better lung function. One study found that a 1SD increase in serum selenium was associated with a 23.7 ml (95% CI 15.0 to 32.5) greater FEV_1 in a sample of the NHANES III survey [96] and a recent British study reported that a 1SD increase in the serum level of the mineral was related to a 52 ml (95% CI 7 to 96) greater FEV_1 in adults [142].

b) Magnesium

Britton *et al.* reported that healthy adults had a higher FEV_1 (27.7ml; 95% CI 11.9 to 43.5) when having an intake of magnesium 100mg/d higher than the mean [143]. This association was observed after adjusting for age, sex, height, effects of atopy and smoking. A second cross-sectional study carried out a few years later in the same population confirmed these associations with an increase of 37.1ml in FEV_1 (95% CI 10.6 to 84.7) in adults having the higher intake of the mineral. This association was independent from the effect of vitamin C [144]. These findings were not confirmed in the study by Hutland *et al*, in which lung function was inversely associated with intake of magnesium [100].

3.2.5.2 Minerals and respiratory symptoms of asthma, BHR or atopy

a) Selenium

At present, there are three observational studies that assessed the relation between dietary intake of selenium and asthma in adults [110-112], with only one showing a beneficial association against risk of asthma in those who were in the highest quintile of consumption [110]. This study is the largest of the three, and included a large number of possible confounders (Table 3.15).

Several studies have assessed blood levels of selenium in asthmatic subjects, reporting lower selenium levels in plasma [145, 146] and whole blood [147] of asthmatic subjects compared to healthy controls. In addition, there is also some evidence of reduced activity of GSH-Px in subjects with aspirin-sensitive asthma [148, 149]. No association was found between serum selenium and atopy in a large community-based study [117].

b) Magnesium

Most of the evidence available from observational studies, suggests that intake of magnesium was not associated with seasonal symptoms of asthma [111], severity of asthma [112], current asthma and asthma ever [106]. In relation to BHR there is insufficient and mixed evidence as one study reported an association between a low consumption of the mineral and being reactive to methacholine [111] but this was not confirmed in the study of Woods and colleagues [106] (Table 3.15).

c) Zinc

A low intake of zinc has been related to a higher prevalence of seasonal symptoms but not BHR in one cross-sectional study [111], while Shaheen and colleagues found a weak negative association between high intake of zinc and asthma [110]. Another two observational studies have found no association between intake of this nutrient and severity of asthma [112], current asthma, asthma ever, and BHR [106] (Table 3.15).

Overall, there are some indications for a beneficial effect of magnesium in lung function, although the number of studies is small. There is yet not enough evidence to suggest that selenium has a beneficial effect on lung function or symptoms of asthma. For zinc, the evidence available provides inconsistent results and due to the small number of studies, more research is needed to estimate the potentially beneficial role of this antioxidant. To conclude, there is limited epidemiological evidence to suggest that selenium, magnesium and zinc may have a beneficial role in lung function and asthma outcomes.

Table 3.15: Association between dietary intake of antioxidant minerals and symptoms of asthma and BHR in adults

Mineral	First author/Type of study	Intake compared	Respiratory outcome	OR (95% Confidence interval) [Adjustment]
Selenium	Shaheen [110] Case-control	Highest vs. lowest quintile (µg/d)	-Asthma	0.56 (0.35 to 0.89) [Age, sex, BMI, social class, housing tenure, employment status, whether a single parent, smoking, passive smoke exposure at home, and TEI]
	Soutar [111] Cross-sectional	Lowest vs. highest tertile (µg/d)	-Seasonal symptoms -BHR	1.64 (0.56 to 4.84) 1.61 (0.51 to 5.08) [Age, sex, and smoking]
	Picado [112] Case-control	Mean intake (mg/d)	-Severity of asthma	75.5± 27 vs. 79±.0. 7 (p>0.05) [Age, sex, and TEI]
Magnesium	Soutar [111] Cross-sectional	Lowest vs. highest tertile (mg)	-Seasonal symptoms -BHR	1.68 (0.57 to 4.94) 5.63 (1.42 to 22.33) [Age, sex, and smoking]
	Picado [112] Case-control	Mean intake (mg)	-Severity of asthma	328.0± 69 vs. 358.0 ± 155 (p>0.05) [Age, sex, and TEI]
	Woods [106] Cross-sectional	Mean intake (mg)	-Current asthma -Asthma ever -BHR	1.46 (0.54 to 3.96) 0.98 (0.42 to 2.25) 1.39 (0.58 to 3.30) [Age, sex, smoking, BMI, region of birth, family history of asthma, and TEI]
Zinc	Soutar [111] Cross-sectional	Lowest vs. highest tertile (mg)	-Seasonal symptoms -BHR	4.70 (1.33 to 16.53) 1.90 (0.53 to 6.82) [Age, sex, and smoking]
	Shaheen [110] Case-control	Highest vs. lowest quintile	-Asthma	0.60 (0.36 to 1.02) [Age, sex, BMI, social class, housing tenure, employment status, whether a single parent, smoking, passive smoke exposure at home, and TEI]
	Picado [112] Case-control	Mean intake (mg/d)	-Severity of asthma	10.3±. 3.4 vs. 11.3±. 3.6 (p>0.05) [Age, sex, and TEI]
	Woods [106] Cross-sectional	Mean intake (mg)	-Current asthma -Asthma ever -BHR	1.28 (0.47 to 3.45) 1.01 (0.44 to 2.36) 1.43 (0.60 to 3.41) [Age, sex, smoking, BMI, region of birth, family history of asthma, and TEI]

3.2.6 Flavonoids and the molecular basis

Flavonoids are powerful antioxidants widely distributed in fruits and vegetables (Table 3.16) [150]. They can act in three ways to prevent oxidative stress. Firstly, they have the capacity to be direct scavengers of free radicals, transforming ROS into more stable, less-reactive radicals, the subclasses flavones and catechins being those with the highest antioxidant capacity. Secondly, some of them (e.g. quercetin) are able to chelate iron, thereby inactivating this metal from participating in reactions leading to formation of free radicals and peroxidation (Fenton's Reaction). Thirdly, several flavonoids, but mainly quercetin, are able to affect the activity of one of the enzymes involved in the production of NO, preventing its reaction with ROS and further formation of peroxynitrite, another powerful free radical [150, 151].

It has also been suggested that flavonoids have anti-inflammatory properties, as they are able to inhibit pro-oxidant enzymes that participate in the synthesis of leukotrienes. Specific flavonoids have been shown to reduce complement activation, decreasing the adhesion of inflammatory cells to the endothelium, thus attenuating the inflammatory response [152, 153].

Table 3.16: Main groups of flavonoids and their dietary sources

Class	Compound	Dietary sources
Flavonols	Quercetin Kaempferol	Onion, apple skin, berries, endive, leek, broccoli, grapefruit
Flavanols	Catechin Epicatechin Epigallocatechin gallate	Red wine, tea, apple
Flavones	Apigenin, chrysin, luteolin syricetin, rutin sibelin	Apple skins, berries, broccoli, celery, fruit peels, cranberries, grapes, lettuce, olive, onion, parsley, red wine
Flavanones	Hesperitin, fisetin, narigin, naringenin, taxifolin	Citrus fruit Citrus peel
Anthocyanins	Cyanindin, delphinidin, malvidin	Berries, cherries, grapes, red wine, tea, apple

3.2.7 Flavonoids and the epidemiological evidence

3.2.7.1 Flavonoids and lung function

Only one large population-based cohort study has been carried out, showing a positive association between total intake of catechin, flavonol, and flavones and increase in FEV_1 (regression coefficient 44; 95% CI 18 to 59) in adults who had the highest quintile of intake when compared to those in the lowest [154].

3.2.7.2 Flavonoids and respiratory symptoms

An interest in the effect of flavonoids in relation to their possible protective effect against asthma comes from the evidence of their effects on cardiovascular and oncologic diseases. As the principle is the same, prevention and attenuation of oxidative stress, it may be possible that flavonoids effectively attenuate the inflammatory response seen in asthma. Also, part of the interest comes from the observation that hard fruit consumption (rich in flavonoids) has been negatively associated with prevalence of COPD symptoms and positively associated with lung function [155]. The evidence available so far suggests that intake of apples (rich in flavonoids) is negatively associated with prevalence of asthma [110]. In addition, one study in Finland has examined associations between intake of specific flavonoid groups and asthma suggesting possible protective effects of quercetin, hesperetin and naringenin on asthma [154]. We have recently reported that intake of three major classes of flavonoids was unrelated to asthma or chronic sputum in adults [156].

3.2.8 Food intake and its relation to lung function and asthma

3.2.8.1 Food items and lung function

Fresh fruits and vegetables are common sources of antioxidant vitamins C and E and flavonoids. Several large epidemiological studies have demonstrated that consumption of fresh fruit, fresh squeezed fruit juice and apples has a beneficial impact on FEV_1 [101, 157-159]. Similarly, intake of green vegetables alone or combined with intake of fresh fruit is positively associated with lung function [97, 101, 153] (Table 3.17).

Table 3.17: Association between intake of foods rich in antioxidants and lung function in adults

Food	Food item (Ref)	Intake compared	Outcome of lung function	Difference observed in ml (95% CI)	Adjustment
Fresh Fruits	Fresh fruit [101]	≥ 1 unit /day vs. less than 1 per month	FEV_1	132 (77 to 188)	Age, height, age^2, height^2, BMI, sex, smoking, social class, activity level, and other dietary intakes
	Fresh fruit [158]	Daily consumption vs. rare consumption	FEV_1	188 *	Region, social class, pack-years
	Apples [157]	≥5 units per week vs. none per week	FEV_1	138.1 (58.1 to 218.1)	Age, height, age^2, height^2 BMI, smoking, and TEI
	Citric fruit: orange and grapefruit [157]	≥ 5 units per week vs. none per week	FEV_1	105.4 (-15.6 to 226.4)	Age, height, age^2, height^2 BMI, smoking, and TEI
	Intake of fresh fruit juice and fresh fruit in winter [159]	≥3 glasses per week vs. never drink fruit juice	FEV_1	78 (24 to 132)	Sex, age, height, cigarette consumption, region of residence, household social group
Vegetables	Green leafy vegetables [101]	≥ 1 portion per day vs. none per week	FEV_1	91 (32 to 150)	Age, height, age^2, height^2, BMI, sex, smoking, social class, activity level, and other dietary intakes
Combined items	Healthy diet* * [153]	Daily vs. less than daily	FEV_1	139 (108 to 170)	Age, sex, sex, height, pack year of smoking, BMI, energy intake
	Fruits & vegetables [97]	Above vs. below the median intake	FEV_1 Finland Italy The Netherlands	53 (-50 to 156) 118 (4 to 232) 110 (-4 to 224)	Height, age, smoking, BMI, alcohol consumption, and energy intake

* CI not given

** Healthy diet according to Dutch recommendation: intake of ≥189g/d fruit + ≥45g/d whole grain + 1-30 g/d alcohol vs. ≤189g/d fruit + ≤45g/d whole grain + ≥30 g/d alcohol

Two studies have explored whether these associations are observed longitudinally. The first one looked at the consumption of fruits and lung function at two time points over a period of seven years, finding that a decrease in the consumption of fresh fruit was related to a decline in lung function, while a regular intake did not affect this outcome [158]. The second study reported that an increase in the number of apples eaten in the week over time did not significantly improve lung function when compared with a regular consumption [100]. It may be possible that the beneficial temporal association between fruits rich in antioxidants and lung function is stronger when the subjects keep a regular intake of these foods, and that an increase would not necessarily have an impact in improving a value of FEV_1.

3.2.8.2 Food items and respiratory symptoms

The dietary intake of different types and groups of foods may provide some insights in their relationship with asthma. Some foods are particularly good sources of one specific antioxidant (for example tomato is a good source of β-carotene), while others offer a combination of them (fish are rich sources of vitamins and minerals), so the associations may correspond to different antioxidants. The strongest evidence available comes from fruits and vegetables, as they are well known sources of antioxidants.

Two studies have suggested that an intake of one fresh fruit a day is significantly associated with less risk of wheezing in adults [157, 160]. Similarly, consumption of a regular amount of apples (2-4 units per week) [143] or hard fruits [106] has been negatively associated with current asthma. Positive associations have also been found between intake of vegetables and asthma [106, 161] (Table 3.18).

Limited information is available regarding other food items and asthma. Negative associations have been found between fish, red meat and asthma [106]. Negative associations have been found between intake of rice and bread with BHR and doctor-diagnosed asthma, explained partly by the vitamins contained in the wheat flour [106].

The epidemiological evidence reviewed in this study suggested that a higher intake of vitamin E is associated with greater FEV_1 and FVC as indicators of lung function in the general population, while there is little cross-sectional evidence of an association with respiratory symptoms of asthma. In relation to vitamin C and its dietary sources, there was a consistent epidemiologic evidence of a positive association with a greater lung function. The evidence was far less conclusive in relation to respiratory symptoms of asthma. The epidemiological evidence on total vitamin A, carotenes, or retinol, was suggestive of a positive association with higher FEV_1 in healthy adults, but there is very weak indication of any association with respiratory symptoms or asthma [94, 106, 109, 110, 114].

Table 3.18: Association between intake of fruits and vegetables rich in antioxidants and respiratory symptoms in observational studies

Food	First author	Food or antioxidant	Respiratory outcome	OR (95% confidence interval) [Adjusted for]
Fresh fruit	Butland BK [157]	Intake of one fresh fruit per day versus <1 a week	-Frequent wheezing	0.59 (0.42-0.83) [Sex, smoking habit, social class and salad/ raw vegetable consumption]
	Forastiere F [160]	5-7 fruits per week versus less than one per week	-Wheeze -Shortness of breath with wheeze -Severe wheeze	0.66 (0.55-0.78) 0.68 (0.56-0.84) 0.59 (0.40-0.85) [Sex, study area, father's education, household density, parental smoking, dampness or mould, parental asthma]
	Shaheen SO [110]	Consumption of 2-4 apples per week versus < once per month	-Current asthma	0.68 (0.47-0.98) [Age, sex, BMI, social class, housing tenure, employment status, whether a single parent, smoking, passive smoke exposure at home, TEI)
	Woods RK [106]	Mean intake of apples and pears	-Current asthma	0.83 (0.71 to 0.98) [Age, sex, smoking, BMI, region of birth, family history of asthma]
Vegetables	Hijazi N [161]	Less than two portions of vegetables per day versus ≥ 2 portions per day	-Current asthma	2.83 (0.98-8.09) [Place of residence, nationality, sex, mother's education, family history of asthma, positive skin test, social class]
	Woods RK [106]	Mean intake of green leafy vegetables Mean intake of tomatoes	-Current asthma -Atopy	0.82 (0.67 to 1.00) 0.84 (0.71 to 0.99) [Age, sex, smoking, BMI, region of birth, family history of asthma]

In relation to vegetables rich in antioxidant vitamins, it has been found that mean intake of green leafy vegetables was inversely related to atopy [106], and that eating less than two portion per day of green vegetables would be associated with current asthma in children [161].

3.3 POLYUNSATURATED FATTY ACIDS (PUFA) AND ASTHMA

3.3.1 The biological aspects

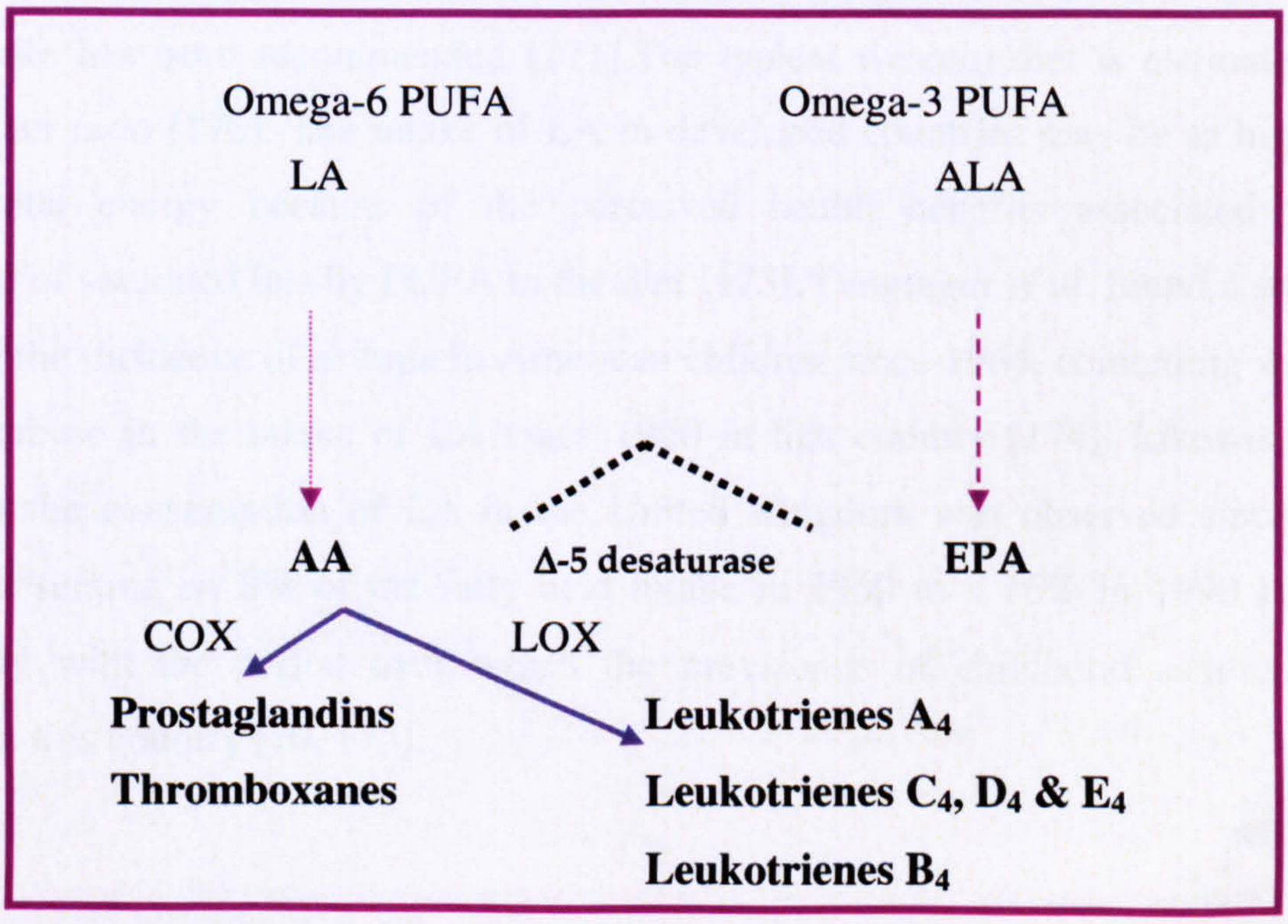
PUFA are long chain fatty acids found in some foods, fish and vegetable oils being the richest sources. Diet also provides several fatty acids denoted essentials, as they cannot be synthesised endogenously. They are linoleic acid (LA; omega-6), and linolenic acid;

(ALA; omega-3). From them, several PUFA are synthesised endogenously, which have structural, metabolic and regulatory functions related to the pathophysiology of asthma [162, 163].

Over the last two decades there has been a change in the type of fats consumed, moving towards an increased consumption of vegetable oils (rich in omega 6 PUFA) and a decrease in the consumption of saturated fats and oily fish [164], the latter being the major source of omega 3 PUFA. In addition, there has been an increase in the consumption of hydrogenated vegetable oil products [165, 166]. These changes in the dietary pattern of fatty acid consumption have been related to the increasing and high prevalence of asthma observed in last twenty years, as suggested by Black and Sharp [167].

The interest in the relationship with PUFA and asthma emerges from the fact that leukotrienes, chemical mediators in the pathogenesis of asthma (bronchoconstriction) [168] can be synthesized from omega-6 PUFA. Leukotrienes are derived from AA, an essential omega-6 PUFA found in cellular membranes. After release from the membrane phospholipids by hydrolysis, two pathways can metabolize AA: through the enzyme cyclooxygenase (COX), which will produce prostaglandins and thromboxanes, or through lypooxygenase (LOX), where leukotrienes are produced (B_4, LTC_4, LTD_4, and LTE_4) (Figure 3.1).

FIGURE 3.1: METABOLISM OF ESSENTIAL FATTY ACIDS



Leukotrienes A_4 and B_4 are potent broncho-constrictors produced by mast cells and other inflammatory cells, including macrophages, monocytes, eosinophils and basophils. They attach to specific target receptors and directly induce bronchial smooth muscle contraction, increase vascular permeability and promote mucus secretion. They also stimulate infiltration of inflammatory cells into airway tissues [168].

As leukotrienes are directly derived from dietary omega 6 PUFA it has been suggested that the composition of dietary fatty acids may alter the capacity to synthesize these lipid mediators of inflammation, thus affecting inflammatory diseases like asthma [168].

Both omega-3 and omega-6 PUFA are metabolised through common pathways, using the same enzymes (Figure 3.1). Thus an increase in the consumption of dietary omega 3 PUFA can inhibit the synthesis of arachidonic acid (AA) from dietary LA [169].Omega-3 PUFA are also capable of acting as inhibitors of the enzymes COX and LOX. When the omega-3 PUFA eicosapentanoic (EPA) and docosahexanoic (DHA) are consumed they can competitively inhibit the formation of prostaglandins and leukotrienes derived from AA, leading to a suppression of neutrophil function and therefore promoting an anti-inflammatory effect [170].

In order to prevent an imbalance of synthesis of the metabolites derived from omega 6, and thus prevent the excess of thromboxanes or leukotrienes, a ratio of omega 6/ omega 3 intake of 2:1 to 4:1, and a total intake of PUFA no higher than 10% of the total caloric intake has been recommended [171].The typical western diet is estimated to have a higher ratio [172]. The intake of LA in developed countries may be as high as 10% of total energy because of the perceived health benefits associated with substitution of saturated fats by PUFA in the diet [173].Yunginger *et al.* found a steady increase in the incidence of asthma in American children since 1964, coinciding with a marked increase in the intake of LA since 1960 in that country [174]. Likewise, an increase in the consumption of LA in the United Kingdom was observed since the 1970s, representing an 8% of the fatty acid intake in 1960 to a 16% in 1990 [171], concurrently with the period over which the prevalence of childhood asthma has increased in this country [70, 175].

3.3.2 The epidemiological evidence

3.3.2.1 Observational studies on intake of fish and fatty acids

At present, most of the evidence from observational studies on intake of omega 3 suggests no beneficial effects of these fatty acids or fish intake in symptoms of asthma or sensitisation to allergens [106, 108, 114, 176-178]. A possible beneficial effect by fish intake on lung function in adults has been reported in three large cross sectional studies [179-181], as well as a greater lung function in asthmatic subjects who eat fish [113]. In relation to sensitisation to allergens, two cross-sectional studies have reported no association between fish intake and specific allergen sensitisation [182], or seasonal rhino-conjunctivitis [177].

There are several studies looking at intake of omega 6 fatty acids, and/or the ratio n6/n3 in adults, and their association with symptoms of asthma. Most of these studies have obtained the information from dietary questionnaires, with few studies assessing these fatty acids in blood. Wakai *et al.* reported that the highest quartile of intake of LA acid in adults was positively associated with rhinitis (OR 1.74; 95% CI 1.09 to 2.77, p per trend=0.02) [177]. Bolte *et al.* reported that higher intake of low-fat margarine was positively associated with current asthma, but intake of margarine was unrelated to hay fever, atopic dermatitis and sensitisation in cross-sectional study on young adults [183]. In the adults participating in the ECRHS survey it was found that intake of margarine was positively associated with hay fever in males but not in females, while intake of butter, margarine and oils showed no association with BHR and atopic eczema [184].

Evidence from case-control studies is mixed. Nagel *et al.* reported that intake of margarine was positively related with physician-diagnosed asthma (OR of highest vs. lowest tertile 1.73; 95% CI 1.05 to 2.87; p per trend=0.05) [178]. Earlier, these authors also reported that intake of oleic acid (omega 9), which can be found in some vegetable oils, was positively associated with hay fever (OR highest vs. lowest quartile 2.86; 95% CI 1.22 to 6.70) [125]. Broadfield and colleagues found that intake of LA did not differ in cases and controls, but erythrocyte membrane's levels of LA were negatively associated with asthma (OR 0.45; 95% CI 0.21 to 0.95) [185]. De Luis *et al.* found that there were no differences in intake of MUFA in cases with asthma compared to healthy controls [113].

With some exceptions [185], a common limitation in most of these observational studies on omega 6 fatty acids and respiratory symptoms of asthma, is that they do not include TEI as a potential confounder. Estimates of the associations with those fatty acids have to be taken with caution, as it may be possible that other dietary sources may not have been measured adequately. This is particularly important in those studies that use margarine or other dairy products as representatives of intake of omega 6 fatty acids, as the proportions of omega 6 and omega 3 fatty acids may have variations.

3.3.2.2 Clinical trials

Some authors have reported that supplementation with PUFA had a deleterious effect in asthmatic adults, based on an increase in the frequency of use of a broncho-dilator when compared to asthmatics that did not eat any fish during the period of study (6 weeks) [186]. Arm and colleagues found no improvement in symptoms of asthma after 10 weeks of supplementation but reported some anti-inflammatory effects such as decreased LT_4 production [187] (Table 3.19).

Table 3.19: Effect of supplemented PUFA on asthma and asthma symptoms in adults

First author	Sample/duration of treatment	Dose supplemented	Respiratory outcomes	Main findings
Picado C [186]	10 aspirin-intolerant asthmatics (mean age 52 yr) 6 weeks control diet (no omega 3) 6 weeks experimental intervention	Experimental intervention: Enriched diet with 150 g sardine meal and capsules of EPA, equivalent to 3 g/d omega 3	-Clinical symptoms -PEF -Bronchodilator use	Experimental vs. control diet: Non-significant increase in severity of symptoms Significant decrease of PEF in weeks 5 and 6 compared to same weeks in control diet. No difference in previous weeks. 13.0 vs. 7.4 puffs per day in week 6
Arm JP [187]	20 asthmatics 12 (10 atopics) received omega 3 (mean age 27 yr) 8 received placebo 10 weeks	Supplementation: EPA 3.2 g/d + DHA 2.2 g/d Or placebo: olive oil	-BHR to histamine -Airways response to exercise (n=5) -Reported symptoms -Neutrophils -Leukotrienes TB_4 (LTB_4) and TB_5 (LTB_5)	Symptoms and respiratory measurements remained unchanged after supplementation. Neutrophils, LTB_4 and LTB_5 production decreased significantly.

Continuation Table 3.19

First author	Sample/duration of treatment	Dose supplemented	Respiratory outcomes	Main findings
Arm JP [188]	17 asthmatics (mean age 26 yr) 9 received omega 3 8 received placebo 10 weeks	Supplementation: EPA 3.2 g/d + DHA 2.2 g/d Or placebo: olive oil	-PEF -BHR to histamine -BHR to inhaled allergen -Use of bronchodilator -Record of symptoms	No significant changes in almost none of the outcomes studied after or previous to treatment with either diet. An exception was a significant attenuation of late asthma response in those treated with omega 3 compared to placebo group.
Thien FC [189]	25 non-smokers pollen-sensitive adults (6 months)	3.2g/d EPA	-BHR -Use of bronchodilator	No significant change in PEF, cough, wheeze, use of medication
Morris A [190]	26 mild-asthmatics 16 weeks	8 weeks diet enriched in omega 6 (n=26) 8 weeks enriched diet with SFA (n=13) or MUFA (n=13)	-FEV_1 -BHR to histamine -PEF -Respiratory symptoms	No significant changes in FEV_1 or PC_{20} when comparing the two diets No changes in use of bronchodilators
Villani F [191]	7 atopic asthmatics (mean age 39 yr) 30 days	3 g/d EPA and DHA	-Airway resistance (R_{aw}) -FEV_1 As outcomes of inhalation challenge with ultrasonically nebulised distilled water	Maximum fall in FEV_1 was -11% after treatment vs. -28% before treatment Maximum increase in R_{aw} was +37% after treatment vs. +26.5% before treatment
Dry J [192]	12 adults (1 year)	3.5 g EPA	-FEV_1	Positive effect on FEV_1 after 9 months of treatment

Other studies found no association between respiratory symptoms and supplementation of PUFA at different doses and lengths of consumption [188-191]. Longer periods of supplementations (1 year) have reported beneficial effects on lung function of supplementation with PUFA in adults [192] (Table 3.19).

Overall, although there is molecular evidence to suggest that a preventive effect in inflammatory response and immune regulation may occur with fish and fatty acids, the

observational and interventional evidence available does not show a clear effect on omega 3 fatty acids intake and asthma and respiratory symptoms.

Conclusion

The biological evidence suggests that oxidative stress might be associated with the mechanisms involved in the asthmatic response. Evidence from experimental studies in laboratory settings suggests that an imbalance between the production of ROS and antioxidant defences leads to activation of inflammatory cells, broncho-constriction and a perpetuation of the inflammatory response.

The antioxidants provided by the diet (vitamins A, C and E, jointly with flavonoids, and enzymatic cofactor minerals) have been shown to have specific antioxidant properties as scavengers, thus reducing the production of ROS and attenuating oxidative stress. They also seem to exert additional protective functions in relation to immune and inflammatory processes. Thus, they have an antioxidant and anti-inflammatory role in the manifestation of respiratory condition.

The epidemiological evidence suggests that many antioxidants have a role in the variability of lung function. Observational studies indicate that a higher intake of dietary antioxidants (assessed as individually or as foods items), especially vitamin E and C, are positively related to lung function. These associations are less convincing for respiratory symptoms and asthma. There is little information on the association between food items rich in antioxidants and asthma, but some studies show they may protect against respiratory symptoms.

Longitudinal studies are also very scarce. Most studies have been cross-sectional surveys, in which exposure and disease are assessed simultaneously, therefore it is not possible to determine whether the exposure preceded or resulted from the disease. To overcome this limitation, prospective investigations of incident asthma, in which both dietary history and some measurements of biological parameters that would support mechanistic links, need to be carried out. Plasma concentrations of vitamins in asthmatic subjects have showed no deficiencies compared to controls in several studies, providing some uncertainties in relation to the effectiveness of this assessment. These

inconsistencies may be caused by differences in the dietary intake, or alterations in the diet in individuals with prevalent disease, and should be interpreted with caution.

The available evidence suggest that the consumption of higher quantities of either fruit or vitamin C from all dietary sources is associated with a small, but consistently higher, level of FEV_1. Likewise, high plasma concentration of vitamin E may confer protection from the development of adult asthma; vitamin C in combination with other antioxidants confers protection against bronchoconstriction associated with ozone exposure, and vitamin C supplementation alone may reduce BHR. The majority of supplementation studies to date have involved single-dose or short-term administration in small numbers of adults, and have focused on asthma control rather than aetiology.

It still remains to be answered whether dietary antioxidants protect against the development of asthma in adults. The assessment of other markers of antioxidant in plasma may offer an additional insight in the relationship between antioxidants, asthma and respiratory symptoms. These markers are defined in the next chapter, where the main markers assessed for the study of oxidative stress in asthma are described, and the epidemiologic evidence reviewed.

CHAPTER 4
Biomarkers of oxidative stress and antioxidant status in asthma

It is accepted that oxidative stress is present either facilitating the manifestation of respiratory symptoms or as consequence of the inflammatory response in asthmatic subjects. There are a number of systems involved in such process, some of them prompting the accumulation of oxidants and thus of the inflammatory response, and others than act trying to counteract such damage. A group of them, dietary antioxidants, were reviewed in Chapter 3, and are known as inhibitors of the oxidative process.

As well as dietary antioxidants, There are a number of other indices that can be assessed to establish the to which extent they are related to the oxidative stress present in asthma. Although there are different criteria to group them, the assessment of such indices is generally based in their composition or in their oxidative or reductive capacity. Chapter 4 aims to give an overview of these additional markers that are currently being use for the assessment of the relationship between oxidative stress and asthma.

The first section of the chapter provides a definition of a biomarker as well as a brief epidemiological review of the most commonly used to determine oxidative stress *in vivo* in relation to asthma. The types of biomarkers are classified according to whether they are products of oxidation (mainly from lipids and proteins); exposure, which includes inhibitors and promoters of oxidative stress; and biomarkers of reductive/oxidative potency of biological fluids. The review is focused in those biomarkers included in the study of this thesis, and also dedicates attention to others that have been used in studies of adult population, but that were unfeasible to assess in the current study, such as malondialdehyde (MDA), nitric oxide (NO), breath hydrocarbons, and antioxidant enzymes.

To analyse the extent to which dietary intake affects biomarkers of oxidative stress in individuals, the second section reviews the relationship that exists between dietary antioxidants and biomarkers of oxidative stress. The chapter concludes with

observations in relation to the advantages and limitations that are currently in discussion between biomarkers, diet and asthma.

4.1 Definition and classification of biomarkers used for the study of asthma

The oxidative damage produced during the inflammatory response of asthma generates a series of ROS that react with more stable molecules, affecting their structure and functionality. The assessment of these oxidants may provide a close idea of the level of oxidative stress that is affecting an individual. However, the direct measurement of ROS is difficult, due to their short life and their condition of highly reactive species. For this reason, oxidative stress has often been measured by assessing the damage that ROS generate on specific molecules.

In 1993 The World Health Organization proposed that a biomarker is a substance that is measured in a compartment within an organism [193]. This broad definition may include three categories of biomarkers. These are biomarkers of effect, which can be recognised as associated with an established or possible health impairment or disease; biomarkers of exposure, defined as measurement of an exogenous substance or its metabolite, or the product of a reaction between an agent and some target molecule or cell; and biomarkers of susceptibility or reductive/oxidative capacity, as indicators of an inherent or acquired ability of an organism to respond to the challenge of exposure to a specific substance.

The analysis of biomarkers is increasingly being incorporated in studies of asthma in order to have a better understanding of the magnitude and relationship between this disease and oxidative stress. Among the most investigated have been markers of nutritional exposure, such as antioxidant vitamins and minerals. Biomarkers of effect are related to the composition of substances produced as result of the reaction between ROS with biological molecules (Table 4.1). As lipids and proteins are the molecules more likely to be oxidised, the products derived from their oxidation are commonly used as biomarkers of effect or oxidative stress in asthmatic subjects, jointly with the products generated as consequence of the airway inflammation [194]. The susceptibility of asthmatic individuals to defend themselves from the damage that an

oxidative imbalance may cause can be measured on several endogenous chemical substances, such as antioxidant enzymes and uric acid.

Table 4.1: Biomarkers used to estimate oxidative damage in asthma

Type of biomarker	Biomarkers used
Effect: products of oxidation ***Lipid peroxidation***	• Isoprostanes • Malondialdehyde (MDA) • Lipid hydroperoxides
Protein oxidation	• Protein carbonyls
Airway inflammation	• Production of Nitric oxide (NO)
Exposure: inhibitors of oxidative stress	• Antioxidant vitamins A, C and E • Minerals Selenium, Zinc, Magnesium • GSH and enzymes GSH-Px, SOD, CAT • Uric Acid
Reductive/oxidative Potency	• Ferric reducing ability of plasma (FRAP)

4.1.1 Biomarkers of effect of oxidative stress

4.1.1.1 Biomarkers of lipid peroxidation

Lipid peroxidation is a characteristic event of the damage that ROS inflict on PUFA. Due to their high vulnerability, they are easily targeted by ROS, initiating a chain of reactions where different products are synthesised. The initial products of lipid peroxidation of PUFA, mainly arachidonic acid, are hydroperoxides, which will follow further decomposition into other substances such as aldehydes, malondialdehyde and eventually isoprostanes. The importance of this process in asthma is that a series of compounds generated as consequence of the lipid peroxidation of AA are related to the activation of inflammatory cells. Therefore, it has been suggested that measuring markers of these products also indicate that inflammation is occurring.

a) Isoprostanes

Peroxidation of membranlipids caused by ROS leads to the non-enzymatic production of a series of prostaglandin-like compounds, namely isoprostanes, which were discovered in 1990 by Morrow *et al.* [195]. The synthesis of isoprostanes can result

from the peroxidation of three PUFA: first, those derived from arachidonic acid (AA), which generates four classes of isoprostanes, of which 8-iso-$PGF_{2\alpha}$ (F2-ip) has been investigated most extensively for its stability; second, those derived from peroxidation of γ–linoleic acid; and third, those generated as consequence of peroxidation of eicosapentaenoic acid (EPA). F2-ip is commonly produced as consequence of the lipid peroxidation generated by ROS released from inflammatory cells in the airways or by most cells types present in the lungs (smooth muscle, epithelium, endothelium, platelets, and inflammatory cells) [196].

The measurement of F2-ip has generated much interest, as they provide a reliable index of oxidative stress *in vivo* [197]. The fact that isoprostanes have a stable structure has facilitated their measurement not only in plasma, but also in urine, broncho-alveolar fluids and breath condensate. The measurement in breath condensate is considered a non-invasive and effective method to assess oxidative stress in asthmatics that allows more direct conclusions on the oxidative stress that occurs in the lungs rather than systemic oxidative stress [195]. In addition, F2-ip is present in relatively high concentrations, and are detectable in healthy individuals, indicating that lipid peroxidation is a continuous process that takes place in physiological conditions [197].

It is recognised that ranges of F2-ip may vary widely in adults, as it is produced regularly under physiological conditions, but increased plasma and urinary levels have been reported in a series of pathologies in which oxidative stress is involved, such as hyper-cholesterolemia [198] and atherosclerosis [199]. Likewise, adults with renal chronic failure requiring haemodialysis have up to three times higher levels of F2-ip than healthy subjects [200]. There is some evidence that smoking increases oxidative stress in teenagers and adults as measured by biomarkers different to F2-ip [201, 202] but the evidence coming from studies assessing F2-ip in healthy smoker adults, shows dissimilar results even when the medium assessed is the same. Block *et al.* found no association between smoking status in adults and their plasmatic levels of F2-ip [203]. Two studies have explored whether urinary levels of F2-ip are associated with smoking condition and if they can be reduced after a short-term supplementation with antioxidants: while Jacob *et al.* reported no associations at all, Reilly *et al.* reported a significant positive association between smoking and F2-ip, and a significant decrease of this biomarker after the antioxidant supplementation therapy [198, 204]. Another

study comparing urinary excretion of F2-ip in smokers and nom-smokers found significant differences in the levels of F2-ip [205]. In addition, Van Hoydonck *et al.* found little or none concentrations of F2-ip in exhaled breath condensate of healthy smokers [206].

The evidence of F2-ip and asthma comes from few studies, which have included a small number of participants. Two case-control studies in adults have reported that asthmatics had between 1.5 to 7-fold higher levels of F2-ip in exhaled breath condensate when compared to non-asthmatic subjects [207, 208]. In line with these findings, a third study found higher levels of plasma F2-ip in adolescent asthmatics when compared to healthy matched controls [209]. Severity of asthma was also significantly associated with higher levels of production of F2-ip in two of these studies (Table 4.2) [207, 209]. Evidence from studies in children also suggests that a higher oxidative stress as shown by higher levels F2-ip is present (Table 4.3). Experimental studies suggest that this may be partly explained by the capacity that F2-ip have to indirectly activate neutrophils, leading to enhanced adhesion to endothelial cells [210]. In addition, other classes of isoprostanes have been shown to be broncho-constrictor agents, and to contribute to non-specific airway hyper-responsiveness [211].

Evidence from RCT suggests that challenge with methacholine increases continuously the urinary excretion of F2-ip in adults, hours after they have had the test, which would imply an ongoing oxidation process [212]. On the other hand, no change has been found in levels of F2-ip in exhaled breath condensate of patients before and after taking different doses of antibiotics for several weeks [213].

One possible limitation on F2-ip is that the evidence accumulated so far, has been essentially limited only to the production of 8-iso-$PGF_{2\alpha}$. Experimental studies have demonstrated that other classes of isoprostanes are equally or more specific and that their production can also have an impact in pathophysiology of asthma [211].

Table 4.2: Evidence of association between oxidative stress as measured by F2-ip and asthma in observational studies in adults

Reference	Design Case-control studies	Characteristics of asthma	Type of F2-IP assessed and Fluid used	Main results
[207]	**Cases:** 12 mild asthma (27.8± 1.34 yr) 17 moderate asthma (47±5.15yr) 15 severe asthma (38.9± 4.2yr) **Controls:** 10 healthy individuals	**Mild asthma:** symptoms twice or less a week, $FEV_1 \geq 80\%$ pred. **Moderate asthma:** daily symptoms, daily use of β2-agonist, FEV_1 between 60 and 80% of expected level **Severe asthma:** continual symptoms, limited physical activity, nocturnal asthma and $FEV_1 < 60\%$	8-iso-$PGF_{2\alpha}$ in exhaled breath condensate (pg/mL)	- F2-ip were detectable in controls (15.8± 1.6) - Mild- and moderate asthmatics had 2-fold l than controls (33.7± 2.8; $p<0.01$ and 38.3 ±3.7; $p<0.001$, respectively) - Severe asthmatics had 3-fold higher levels than controls (49.1± 5.0; $p<0.001$) - No correlation between F2-ip and FEV_1 in any group.
[208]	**Cases:** **31 patients with aspirin-induced-asthma (AIA):** 17 non-steroid (41± 23yr) 14 steroid-treated (42± 28yr) **26 patients with mild-to-moderate asthma and aspirin-tolerant (ATA):** 11 non-steroid (47 ±18yr) 15 steroid-treated (48 ±18yr) **Controls:** 16 healthy individuals (45±17 yr)	- Asthma diagnosed by history of recurrent wheezing and chest tightness - Bronchodilator response >15% increase in FEV_1 with inhaled albuterol - Airway reactivity after histamine challenge with PC_{20} less than 8 mg/ml	8-iso-PGF in exhaled breath condensate (pg/mL)	- No significant difference in levels of F2-ip between the two groups of asthmatics (AIA 90.0 ±19.0 *vs.* ATA 79.2 ±19.5) - AIA and ATA patients had higher levels of F2-ip than controls (131.8± 31.0 and 77.3± 21.9 compared with 21.9± 4.5; $p<0.05$ and $p<0.05$, respectively) - No correlation between F2-ip and pulmonary function in any of the groups of patients.
[209]	**Cases:** 15 asthmatic adolescents (15 yr) 7 Infrequent episodic (IE) 4 Frequent episodic (FE) 4 Persistent (P) **Controls:** 15 healthy controls (14 yr)	Asthma diagnosed by doctor Use of inhaled therapy Improvement ≥12% in FEV_1 in response to bronchodilator. **Severity of asthma:** **IE:** <6 exacerbations/ week **FE:** 4-6 exacerbations/ week, use of bronchodilator <3 times a week **P:** symptoms most of the days, use of bronchodilator most of days, preventative therapy always required.	Total 8-iso-$PGF_{2\alpha}$ in plasma (pg/mL)	Levels of F2-ip: 213 (IQR 122-455) *vs.* 139 (109-174) p= 0.042 No correlation between total 8-iso-PGF levels and FEV_1 Level of 8-iso-PGF positively associated with inhaled corticosteroid use in asthmatics (p= 0.027) F2-ip increased significantly with the severity of asthma (p=0.027)

Continuation Table 4.2

Reference	Design	Characteristics of asthma	Type of F2-IP assessed and Fluid used	Main results
[214]	11 mild atopic (21 to 44 yr) underwent inhaled allergen challenge	Allergen inhalation challenge tested to cause a 20%or greater fall in FEV_1. Lung function assessed in baseline, every15 minutes after challenge and then hourly for 8 hours.	Un-metabolised F2-ip assessed as: Urinary excretion ng/mg creatinine	Excretion of F2-ip was significantly increased after 2 hours allergen inhalation and remained elevated in all urine collections for the following 8 hours.
	9 mild allergic asthmatics (24 to 46 yr) underwent a bronchoscopy.	Bronchoscopy with bronchoalveolar lavage performed before and after allergen instillation.	Released to bronchial alveolar lavage fluid (BALF) pg/mL	Significant release of F2-ip into the BALF precipitated by allergen challenge compared to baseline.
	All participants were positively skin tested to at least one allergen	4 volunteers underwent methacholine challenge	Urinary excretion ng/mg creatinine	No alteration in excretion of F2-ip in those that had methacholine challenge compared to baseline values.
		6 volunteers underwent pre oral treatment with aspirine (900 mg previous day and repeated on day of study) previous to allergen challenge	Urinary excretion ng/mg creatinine	Significant increased excretion after intake of aspirin.
[212]	9 non-smoking mild atopic asthmatics 921-44 yr)	Provocation with threshold allergen challenge causing a fall in the FEV_1 of 20% or greater 4 volunteers underwent methacholine challenge	Urinary excretion of 15-F_{2t}-Isop (8-iso-PGF_2) (ng/mg creatinine) Measured every 2 hours during 8 hours after challenge with allergen or methacholine	Excretion of 15-F_{2t}-Isop was significantly increased at all times after allergen challenge compared to basal conditions Methacholine challenge did not cause any significant alterations in the urinary concentration of 15-F_{2t}-Isop
[215]	71 stable asthmatics 23 asthmatics with acute exacerbation 23 subjects with bronchiectasis 29 healthy controls	Clinical asthma pattern categorised according to GINA	Concentration in induced sputum 8-iso-$PGF_{2\alpha}$ (ng/L)	Median (IQR): Healthy controls: 1234 (41-290) Stable asthmatics: 216 (103-389); p=0.029 vs. healthy subjects Bronchiectasis: 698 (264-1613); p<0.0001 vs. healthy controls; p<0.0001 vs. stable asthmatics.

Table 4.3: Evidence of association between levels of F2-ip and asthma in children

Reference	Design	Characteristics of asthma	Type of F2-IP assessed/ Fluid used	Main results
[216]	**Cases:** 14 stable mild persistent asthmatic and ICs naïve (9.3 ±0.9 yr) 13 stable mild-to-moderate asthmatic + ICs (11.3±0.8 yr) 9 unstable asthmatic (12.2±1.2 yr) **Controls:** 19 healthy children (10.0±8 yr)	**Stable asthma**: minimal need for short-acting β2-agonists, no exacerbation, and no use of systemic steroids in the last 3 months. **Unstable asthma**: children required one or more urgent-care visits for asthma and more than 3 oral steroid bursts in the last year, had been taking high-dose ICs and long-acting β2-agonists for at least 3 months **Ics naïve**: had not been treated with ICs for at least 1 month	8-Isoprostane Exhaled breath condensate (EBC) pg/mL	No difference in levels isoprostanes in EBC between the three groups of asthmatics, but each group significantly higher than controls: Median (Interquartile range, IQR): ICs naïve: 16.2 (11.7 to 19.1) p<0.001 ICs-treated/stable asthma: 18.1 (14.8 to 20.5) p<0.001 Unstable asthma: 29.7 (4.8 to 35.1) p<0.01 Control: 3.5 (2.6 to 7.9)
[217]	12 asthmatic children aged 8-15 yr sensitized to house dust mite (HDM) 3 months living in a Residential Home at high altitute, unexposed to HDM	All children with history of mild bronchial asthma. None had received ICs for at least 2 months	8-Isoprostane EBC (pg/mL)	Levels of F2-ip at initial time of living in high altitute vs. end of period decreased significantly: 17.5±3.2 vs. 7.4±3.3; p<0.003
[218]	**Cases:** 12 ICs naïve asthmatic (10±0.8 yr) 30 ICs with mild to moderate persistent asthma (11±0.6 yr) **Controls:** 12 healthy children (9±0.5 yr)	ICs treated children had low to medium doses a t a constante dose for at least 2 months (300 µg/d average dose)	8-Isoprostane EBC (pg/mL)	No statistically significant difference in levels of isprostanes in the two groups of asthmatic children, but each was significantly higher than that found in controls: Mean±SD (95% CI): ICs naïve: 56.4±7.7 (39.4 to 73.4) p<0.01 ICs treated: 47.2±2.3 (42.4 to 52.0) p<0.05 Controls: 34.2 ±4.5 (24.3 to 44.0)

Continuation Table 4.3

Reference	Design	Characteristics of asthma	Type of F2-IP assessed/ Fluid used	Main results
[219]	**Cases:** 15 atopic asthmatic with acute asthma exacerbation (11 yr, range 6-16) **Controls:** 10 healthy children (10.1 yr, range 4-14)	Asthma exacerbation was defined as increasing signs and symptos of asthma (coughing, wheezing, shortness of breath) unresponsive to the patient's routine asthma medication and additional β2–agonist therapy.	8-Isoprostane EBC (pg/mL)	Statistically significant higher values of isoprostanes in asthmatic versus helthy children: Median (IQR): 12.0 (9.4-29.5) vs. 2.6 (2.1-3.0) p<0.001
[220]	**Cases:** 20 atopic non asthmatic (10±0.8 yr) 30 atopic, ICs naïve with mild-intermitent asthma (9±0.7 yr) 25 atopic, ICs treated, stable mild-to-moderate persistent asthma (10±0.6 yr) **Controls:** 20 healthy children (9±0.5 yr)	Atopic children had a history of atopy and positive skin test presults. All had allergic rhinitis. ICs naïve children had symptoms less than twice a week, FEV_1 80% or greater of predicted value, 12% reversibility to salbutamol, or positive provocation to methacholine or exercise. ICs treated were receiving low-to-medium doses of inhaled corticosteroids for at least 2 months (300 μg/d average daily dose)	8-Isoprostane EBC (pg/mL)	Cases with asthma, but not those with atopy only, had significantly higher levels of F2-ip than healthy children. Medians (IQR): Atopic non asthmatic: 15.8 (13.9-20.1) p>0.05 ICs naïve asthma: 29.8 (26.0 –34.3) p<0.001 ICs treated asthmatic: 33.0 (28.5 –35.8) p<0.001

b) Malondialdehyde (MDA)

Determination of MDA through the assay Thiobarbituric acid-reactive substances (TBARS) was one of the earliest assessments of oxidative stress in humans [221]. The method is based on the measurement of concentration of lipid peroxides, mainly MDA, after their reaction with TBARS. MDA is an end product of PUFA containing three or more double bonds. TBARS is commonly assessed in plasma, red cells and low- and very-low density lipoproteins. The method is considered an overall approach to systemic oxidative stress with simplicity, low cost and rapidity, which have prompted the use of this marker in the last 20 years as the commonest index of lipid peroxidation in acute and chronic diseases in which oxidative stress is involved [222-228].

TBARS has also been used in the study of oxidative stress and asthma, with mixed results. Elevated levels have been observed in plasma of asthmatic adults [229-231], but other authors have found no differences in levels of MDA in asthmatics compared with healthy subjects when assessed in plasma [123, 232] or exhaled breath condensate [233]. It has also been suggested that healthy individuals with normal lung function have lower levels of oxidative stress as mirrored in a significant negative correlation between TBARS and FEV_1 [234].

The inconsistence of these results has been attributed to several limitations of the assay. TBARS is considered a non-specific marker of lipid peroxidation [222]. An example of this is given by a study that demonstrated the low specificity of the assay by simultaneously measuring F2-ip and MDA. It was found that the time-course of formation of both markers was highly correlated, indicating that lipid oxidation was occurring. However, values of MDA were much higher than those for F2-ip [38]. This is explained by the fact that TBARS captures several other compounds than MDA that are also reactive toward thiobarbituric acid, including sugars, amino acids, and bilirubin.

Another limitation of the test is that it does not measure the free MDA content of the lipid oxidation but rather measures MDA generated by decomposition of lipid peroxides during the acid-heating stage of the test, the oxidation produced as part of

the laboratory technique [235]. In order to improve the specificity of the assay, the conventional technique of gas chromatography/mass spectrometry has been replaced by other methods such as high performance liquid chromatography and fluorometry. It is thought that through these assays it is possible to detect up to 90% of the MDA formed by lipids that underwent oxidative stress. In spite of this, the doubts about its usefulness remain under debate [235].

c) Breath hydrocarbons

Pentane and ethane are volatile compounds synthesised from the oxidation of omega 6 and omega 3 fatty acids, respectively. They are released into the breath and their measurement on exhaled breath condensate is a non-invasive method to assess oxidative stress and inflammation in the airways. The evidence published so far suggests that levels of hydrocarbons are significantly increased in asthmatic subjects [236, 237].A main limitation of this assay is the high likelihood of error of measurement due to the risk of contamination with ethane and pentane present in the air of the environment [238].

4.1.1.2 Biomarkers of protein oxidation

a) Carbonyls of proteins

Proteins represent the second most vulnerable target for oxidation after PUFA. ROS can attack the side chains of the protein, leading to formation of new products, loss of functionality and irreparable destruction in some cases [239]. In the presence of O_2 all amino acid residues of proteins are susceptible to oxidation by ROS, •OH being the commonest aggressor. Depending upon the amino acid residue that has been attacked different compounds will be synthesised, which can be used in some cases as markers of oxidative stress-mediated damage in proteins.

Carbonyls are commonly generated by several pathways as a consequence of the reaction between amino acids and ROS. The presence of carbonyl groups in proteins has been used as a marker of ROS-mediated protein oxidation.

In humans it has been found that carbonyls are elevated in plasma of individuals with acute respiratory distress syndrome [240], in infants with chronic lung disease [241], and in tracheal aspirates [242]and plasma [243] from preterm babies with a birth-weight of <1,500g. In healthy smokers, higher levels of carbonyls in plasma have been found when compared to non-smokers [244].

The evidence of use of carbonyls as biomarkers of oxidative stress in asthmatic adults was found a few years ago and a small number of studies have accumulated (Table 4.4). Foreman *et al.* found that oxidation of proteins was significantly elevated in asthmatics after administering an allergic challenge [245]. They suggested that the oxidation was largely accounted for the recruitment and activation of eosinophils as seen in the high positive correlation between them and carbonyls. The other two studies in adults compared concentration of carbonyl proteins in asthmatics with healthy controls. Aldridge *et al.* found no difference in the production of carbonyls in sputum of stable asthmatics when compared to healthy controls [246]. In contrast, Nadeem *et al.* found that plasma carbonyls produced by adults with bronchial asthma were significantly higher than those found in healthy age-matched adults [229].

Two of the studies reported that carbonyls correlated positively with the production of eosinophils amongst the asthmatics [229, 245]. One possible explanation is that eosinophils may be responsible for the majority of ROS generated in the airways in asthma. The fact that the assessment was made in broncho-alveolar lavage or sputum allows the observation that the production of both carbonyls and eosinophils directly account for the inflammatory response in lungs, and that it is not just an indicator of general oxidative stress as it may be considered when assessed in plasma. Also, two of the studies reported that there was a non significant correlation between FEV_1 and the concentration of carbonyls in sputum [246] or in plasma [229].

Table 4.4: Epidemiological evidence of oxidative stress in asthma as measured by protein carbonyls

Reference	Design	Diagnosis of asthma	Fluid/tissue Assessed (units)	Results
[245]	12 asymptomatic atopic asthmatic (25.6±1.0 yr) Allergen challenge vs. Sham-challenge Measurement of carbonyls 18hr later	Patients had no treatment other than rescue β_2-adrenergic drugs, discontinued a day before the study.	Broncho-alveolar lavage nmol/mg protein	Increase of carbonyls production 18 hr after challenge when compared to a saline challenge (0.82 vs.0.23; p<0.01)
[246]	51 adults (mean 42.3yr) 24 healthy controls (mean 37.4yr)	Clinical history of asthma as defined by the American Thoracic Society All subjects clinically stable, with no history of asthma exacerbation in the last 4 weeks	Sputum supernatant nmol/mg protein	No correlation between carbonyls and FEV_1 in asthmatics No significant difference in levels of carbonyls when the two groups were compared.
[229]	38 adults (15-40 yr) with bronchial asthma: Mild (M) Moderate (Mo) Severe (S) 23 age-matched healthy controls	- Recurrent symptoms of breathlessness and wheezing - Improvement ≥12% in FEV_1 in response to bronchodilator	Plasma nmol/mg protein	Total asthmatics vs. Control: 1.28± 0.5 *vs.* 0.8± 0.03 (p<0.0001) M: 1.2 ±0.04; Mo: 1.22± 0.08; S 1.43± 0.09 (p>0.05) Negative but non-significant correlation between carbonyls and degree of airways obstruction (predicted FEV_1%) (r=-0.33)
[247]	32 asthmatics with acute exacerbations 97 stable asthmatics		Plasma nmol/mg protein	No differences in levels of stable vs. asthmatics with exacerbations.

The assessment of carbonyls seems a reliable way to estimate oxidative stress in humans, although so far in asthma has been limited. It is difficult to decide whether these results may be extrapolated to population-based studies, in which a large number of healthy people are included. So far, the groups studied were very small and the assessment of bronchial lavage fluid is not feasible in epidemiological studies.

As the various biomarkers of lipid oxidation represent concentration of products generated by reactions between PUFA and ROS, there has been interest in evaluating to which extent these biomarkers are related to each other. There is some experimental evidence suggestive of a linear correlation between MDA and F2-ip after oxidative stress is induced [248]. To date the scarce published evidence of correlation between MDA and F2-ip in adults, shows that they were very weakly correlated to each other (r=0.13; p=0.05) [203].

More but conflicting evidence is available regarding the correlation between MDA and protein carbonyls. One study showed a good correlation between MDA and carbonyls of proteins as assessed in serum of healthy adults that underwent intense exercise (r=0.92; p=0.01) [249], while another showed borderline correlation when assessed in plasma of healthy adults (r=0.65; p>0.05) [250]. A third study found no significant correlation between these biomarkers when assessed in bronchoalveolar fluid in newborn babies (r=0.2; p>0.05) [251].

4.1.1.3 Biomarkers of airway inflammation

a) Nitric oxide

NO is synthesised by several types of pulmonary cells in the body, including inflammatory, endothelial and airway epithelial cells [252], with the highest production coming from macrophages and epithelial cells. As inflammatory cells can activate the inducible enzyme responsible for the synthesis of NO, this gas has gathered much attention as a biomarker of oxidative damage in asthma [253].

NO has physiological and pathological functions, depending if is synthesised either by endothelial (eNOS) and neuronal nitric oxide synthases (nNOS), or by inducible nitric oxide synthases (iNOS) respectively. The latter enzyme is expressed by epithelial and inflammatory cells, which are found in much larger amount in asthmatic subjects than healthy individuals [254]. The physiological effects of NO include its action as vasodilator tone in blood pressure regulation, neurotransmission, and regulatory functions of the respiratory and gastrointestinal systems. When NO is induced by iNOS, its production is much grater than that observed from the other

enzymes. Therefore, it is thought that high levels of NO are indicative of inflammatory response [253].

In addition to its condition as a marker of oxidative stress in the airways, it has been demonstrated that NO has the ability to react with ROS such as superoxide anions to form other potentially harmful reactive nitrogen species, thus acting as a pro-inflammatory agent [255].

The literature investigating the role of NO in asthma is extensive. Numerous studies have demonstrated that NO is elevated in exhaled breath condensate of asthmatic adults [213, 256, 257] when compared to healthy controls. It has also been suggested that exhaled NO offers a sensitive marker not only of oxidative stress and inflammation, but also a useful non-invasive way of monitoring the severity of the disease [253].

Most of the evidence regarding production of NO and asthma has been obtained through exhaled breath condensate. This is non-invasive and considered an accurate method to assess the amount of NO released from the airways. Due to its short life, measurement in other compartments can only be made on its sub-products, such as nitrites and nitrates. It has been found that they are also reliable indices of inflammation and oxidative stress when assessed in plasma [251].

There have been indications that NO decreases significantly in asthmatics on medication. A study reported a significant reduction in exhaled NO after 6 hours following a single high dose of nebulised budesonide in symptomatic moderate asthma, or within 2-3 days after high doses of inhaled corticosteroids [213]. A gradual reduction in exhaled NO has also been observed during the first week of regular treatment of asthma exacerbation, with maximal effect between 3 and 4 weeks [258]. The reduction in exhaled NO has been shown to be dose-dependent when low doses of inhaled corticosteroids are used in patients with mild or moderate asthma [259].

The use of NO in exhaled breath condensate as marker of oxidative damage in asthmatic subjects is expanding. As well as being non-invasive it has high sensitivity,

as in asthmatics its presence is largely due to the activation of enzymes released from cells involved in the inflammatory response. Furthermore, it has been observed that the measurement of NO is related to airway reactivity and atopic status [260]. The findings of NO as an accurate marker of oxidative stress in asthma are promising, and are offering a practical, non-expensive and reliable way to obtain information on the oxidative damage involved in asthmatic individuals.

4.1.1.4 Biomarkers of reductive/oxidative potency: The FRAP assay

The body contains several antioxidants to protect against oxidative damage. These are usually referred as endogenous antioxidant defences. Generally, antioxidant nutrients are considered exogenous defences because they are provided by diet. In blood and cells is possible to find several antioxidant enzymes, largely responsible for the destruction of ROS or at least their transformation into molecules that are more stable and inoffensive.

In the plasma, many of these antioxidants are circulating, so is thought that measurement of groups of these antioxidants may be an indirect biomarker of oxidative stress in the body.

There are several assays available to assess '*antioxidant capacity of plasma*', or '*total antioxidant capacity in plasma*'. They estimate the antioxidant capacity of the plasma of different groups of antioxidants [261]. In general, in plasma the activity of resistance to circulating ROS, bilirubin, uric acid, and the activity of some dietary antioxidants such as ascorbic acid, α-tocopherol, and flavonoids can be assessed.

The Ferric reducing ability of plasma (FRAP) is one of these assays, and it is usually referred to as '*antioxidant capacity of plasma*'. FRAP is considered as a potency test that asses the reductive capacity of the plasma. Its determination is based on the measurement of the reducing ability of specific reductants, this is, non enzymatic antioxidants capable of reducing an oxidant [262]. The reaction is known as *redox reaction*, in which an oxidant is reduced at the expense of the oxidation of the reductant. In the FRAP assay, the reductant capacity of bilirubin, vitamin C and E,

flavonoids and uric acid can be detected. This is explained by the fact that the technique uses Fe^{+3} as reagent, so all the antioxidants with the capacity to act as reductants or iron chelators will be detected [263].

Over the last decade there has been increasing interest in using FRAP to estimate the antioxidant capacity of individuals with chronic diseases, including obesity [264] and chronic renal failure [265]. So far, there has been only one study relating FRAP to a pulmonary disease, and it was carried out in neonates undergoing cardio-pulmonary bypass [266]. There is no current evidence regarding the antioxidant power assessed through FRAP in asthma in humans or in animal models of respiratory inflammation.

In spite of the lack of epidemiological evidence regarding FRAP and asthma, several features make it a suitable indicator of antioxidant power in plasma. Firstly, there are technical advantages, as the FRAP assay gives fast, reproducible results with plasma, with single antioxidants in pure solution and with mixtures of antioxidants in aqueous solution added to plasma. The technique has a relatively low cost and is considered of low complexity. FRAP is also provides information of the activity of vitamins C and E, thus offering an approach to the reductant capacity they have. It has been demonstrated that FRAP correlates positively and significantly with plasmatic levels of ascorbic acid and tocopherol [262].

The assessment of FRAP also estimates the value of polyphenols as demonstrated by Schlezier *et al.* In the study of nutritional risk factors for asthma they represent one of the potent reductants and iron chelators available in foods [263]. The assessment of flavonoids individually or as a group in plasma involves technical and economic difficulties that are partly suppressed by the assessment of FRAP.

4.1.3 Biomarkers of exposure: inhibitors of oxidative stress

4.1.3.1 Antioxidant Enzymes

The enzymatic antioxidants include the families of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px).

SOD is the primary extra-cellular enzyme, and it is the most highly expressed enzyme in lungs. It has been suggested that SOD might play a role as both an antioxidant and a regulator of the signalling of several inflammatory cells, such as eosinophils, neutrophils, and macrophages [80].

Antioxidant enzymes reflect current antioxidant status, which embraces some limitations for studying the aetiology of asthma. Levels of these enzymes as measured in plasma or broncho-alveolar fluid give a partial view of the effect and intensity that oxidative stress may be causing. Antioxidant enzymes deficiencies have been frequently reported in asthmatics, although there are mixed results. These differences may be partly explained by the fact that different analytical techniques and fluids are being assessed and there are no studies of correlation or comparability between them.

The enzymes SOD and GSH-Px participate in the removal of some oxidants, to reduce them and transform them into water and other stable molecules. The activity of SOD has been reported as increased [267], decreased [251] or unchanged [268] in asthmatics compared to controls. Similarly, the activity of GSH-Px has been reported as decreased [251] and unchanged [269] in asthmatics when compared to healthy individuals.

4.2. DIETARY INTAKE AND ENDOGENOUS LEVELS OF BIOMARKERS OF OXIDATION

The possible effect that dietary intake may exert against chronic non-transmissible diseases has been largely explored. Biomarkers of oxidative stress *in vivo* have emerged as a tool to identify whether oxidative stress is present in those illnesses and as indicators of the intensity of damage. Thus there has been an increasing interest to clarify whether diet and its specific components are able to affect oxidative damage *in vivo*, by modifying the levels of biomarkers, and to which extent these markers remain unchanged by the temporal influence of diet.

Efforts have been gathered to determine whether the effect of a dietary antioxidant alone or combined with others may be similar in diminishing oxidative damage, or if there is any synergic action when several antioxidants are administered, or if there is

no change at all. If the intake of a dietary antioxidant does not change the levels of a specific biomarker, reflect damage inflicted by compounds and products synthesised during oxidative stress, and which are not attenuated by dietary antioxidants. Alternatively, if consumption of an antioxidant does change the levels of a biomarker, it may indicate the capacity that the former has to constrain oxidative damage.

The information obtained from FFQs does not provide certainty on the amount of antioxidants that will truly be available to act once is absorbed. An extensive number of studies have tried to address this point, by estimating through assessment of biomarkers how effective dietary antioxidants can be in the prevention or attenuation of oxidative stress (Table 4.5).

4.2.1 Fruits and vegetables

The investigation on the effects of fruits and vegetables on markers of oxidative stress has included FRAP, F2-ip, protein carbonyls and TBARS. Most of the evidence comes from small samples of healthy adults or 'healthy smokers'. There are no studies on the effect of specific dietary intake of fruits on the levels of biomarkers in asthmatics so far.

It has been shown than supplementation with 200ml/d of tomato soup over a week increases the levels of FRAP. This increase was greater when olive oil was added to the soup. The authors attributed these changes to the content of lycopene and tocopherol of the supplementation [270]. In contrast, another group reported that intake of tomato juice did not affect FRAP in young males, but reduced significantly the levels of TBARS after two weeks of treatment when compared to basal conditions [271].

Supplementation of a daily antioxidant-rich burger plus an antioxidant-rich juice, equivalent to 7 daily portions of vegetables and fruits, did not exert any change on levels of plasma 8-EPI-$PGF_{2\alpha}$ in smokers after three weeks of treatment [272]. The authors reported no differences between those supplemented and the placebo, or within the groups when compared to baseline values. Likewise, production of

protein carbonyls remained almost equal in those supplemented compared to the placebo.

Table 4.5: Summary of evidence regarding the effect of consumption of foods on markers of oxidative stress and antioxidant capacity in urine (U) or plasma (P)

Food Item (ref)	F2-IP		TBARS (MDA)		Protein carbonyls		FRAP
	U	P	U	P	U	P	
Fruits & vegetables							
Tomato soup [270]							+
Tomato juice [271]				-			Null
Rich-antiox. Juice+ Rich-antiox. Burger [272]		Null				Null	
Garlic [273]	–	–					
Cream (powder) legumes and grains [274]	–						
Foods rich in PUFA							
[275]				+			
[276]				+			
[277]				+			
Olive oil [278]				Null		Null	Null
Beverages							
Tea [279]	Null						
[280]	Null						
[281]							Null
Tea with milk [282]							+
[283]							Null
Tea without milk [284]							+
[283]							+
Red wine [284]							+
[285]							Null
[286]				Null			

Null= No effect -= decrease in the level of biomarker assessed += increase in the level of biomarker assessed blank= No information available for that food and biomarker

Garlic is considered to have antioxidant properties due to its flavonoid content [287]. When healthy non smokers adults received a supplement containing a concentrate of garlic, equivalent to the flavonoids provided by 5 apples and 50g of strawberries, over two weeks, it was observed that levels of plasmatic and urinary F2-ip decrease by 29% and 37% respectively, in relation to the basal levels [273].

Whole grains and legumes are rich sources of antioxidants such as phytochemicals, phenolic acids and flavonoids [288, 289]. The supplementation with a powder supplement containing whole grains, beans, legumes, seeds and brown rice during 16

weeks, decreased by almost a third the amount of urinary 8-Epi-$IP_{2\alpha}$ in men diagnosed with a coronary artery disease [274].

4.2.2 Foods rich in PUFA

Results from *in vivo* studies in humans show that supplementation with PUFA significantly increased lipid peroxidation measured as TBARS in male smokers [275], menopausal women [276, 277], and young healthy women [276]. In contrast, supplementation with olive oil did not affect levels of TBARS and carbonyls, nor FRAP in moderately heavy smoker adults [278]. This allows the observation that adults with a higher intake of PUFA may have higher levels of lipid peroxides, derived from a higher oxidation rate.

Conclusion

There is extensive evidence that oxidative stress is a central element of the inflammatory response that occurs in asthmatic subject. Oxidative stress can be measured from the composition of body fluids or specific products of oxidation, in different compartments and conditions. Although biomarkers can be grouped into categories according to whether they measure overall antioxidant capacity, effect of oxidation or susceptibility to be oxidised, the results they give can provide with different interpretations. This suggests that assessing only one biomarker is not enough to conclude that a given oxidant/antioxidant status is present in an individual, but contrary, may offer a partial view of the extent to which oxidative stress has affected. Thus, assessment in an individual may require a combination of methods that will represent different sides of oxidative stress.

Alone, none of these methods can be considered as the most appropriate criterion to evaluate oxidative stress, but the contrary, measuring at least two of them offers a better understanding. Some biomarkers widely used for determining oxidative stress in pathological or acute conditions of oxidative stress are increasingly being used in combination with newer indices intended to be more specific. In relation to asthma, most of the evidence has been gathered around plasma levels of MDA and antioxidant enzymes, while other methods such as F2-ip and carbonyls of proteins are being recently introduced to add to the information these markers give.

Population based studies assessing these markers in asthmatic subjects are still rare, and a limitation arises, as most of the available evidence comes from studies that included a very small number of participants.

Increasing evidence suggest that new biomarkers such as the measurement of F2-ip seem reliable to assess oxidative stress, although this may not be reflected in adults who have a general good health status, as proven by the contradictory evidence found in smokers. Thus, it may be possible that young adults with moderate or mild asthma symptoms may have levels of F2-ip similar to those reported in healthy individuals.

The review of the relationship between dietary intake and the biomarkers included in the current study is inclined towards a scarce, if any, impact on the production of end-products of oxidative stress, as derived from oxidation of PUFA and proteins. It seems that F2-ip remain stable under most of the physiological conditions in which it has been evaluated. This may contribute to consider it as a marker of oxidative stress during illness, but may not indicate oxidative damage in general population with a healthy condition. It also suggests that different dietary habits within young adults with relatively good general health conditions may not be reflected in these biomarkers.

A higher intake of dietary antioxidants may not influence end products of oxidation, but it may affect other biomarkers that reflect the general antioxidant potency of the organism, such as FRAP and antioxidant enzymes. This may be explained on the basis that these biomarkers are the first barrier to ROS thus participating in rapid oxidative/reductive reactions that will constrain further oxidative damage. A decrease in FRAP might be interpreted as a depletion of the defence, or as a higher activity, so either result may be favourable in terms of the antioxidant barrier that is taking place in plasma. Tea appears with or without milk may effectively provide with antioxidants that will contribute to an increase in the FRAP, depending on the quality and variety of the leaves. Chileans drink high amounts of this beverage, replacing in lower socio-economic stratum the consumption of milk during breakfast and teatime. Therefore, the effect that the regular consumption of tea may have

against oxidative stress in this population could contribute to the current knowledge of its relationship with biomarkers.

Overall, the current evidence offers limited information regarding to which extent biomarkers are good indicators of oxidative stress in epidemiological setting with large number of participants. It seems clearer that in the presence of pathologies may confer additional insights into the intensity of oxidative damage that may takes place.

As described in the next chapter, the participants of this study where young adults that live under general good conditions of health and well-being. Several biomarkers of oxidative damage and antioxidant status were measured in order to establish the type of association that would exist between them and respiratory outcomes.

CHAPTER 5
Methodology

5.1 CHARACTERISTICS OF LIMACHE

Limache is an agricultural area of 50,000 inhabitants situated in the Central valley of Chile, 141km from the country's capital Santiago, and 31km from Valparaiso, the regional capital (Figure 5.1). The educational and civic activity of the population is concentrated in the central area of Limache. The configuration of the locality also comprises an outer area surrounded by hills where a considerable part of the population also resides (Figure 5.2).

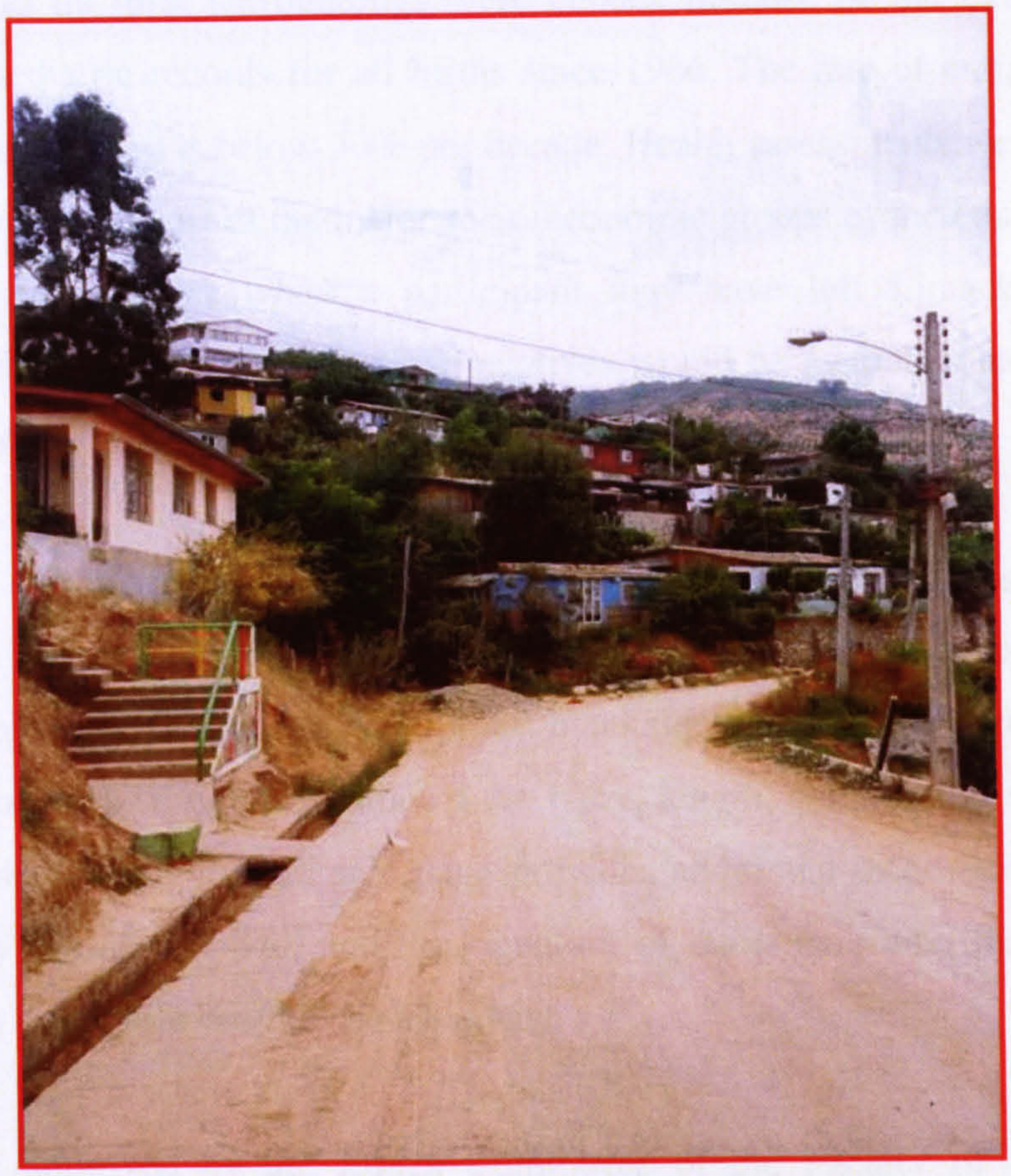
Access to basic services such as tap water and electricity reach a large majority of the area, the rural being slightly less supplied. An idea of the combined sense of rural and urban aspects of Limache can be appreciated in Figure 5.3. In the town of Limache there is a local hospital and several primary and secondary state schools [290, 291]. Poverty in this area is similar to the national mean, with an estimated 18% of the population classified as poor [292, 293].

Historically, Limache has been characterised for its agriculture, which is the main economic activity in the area. A third of its cultivated area is used for the production of vegetables, a large proportion of which is sold in Santiago, or exported. Tomato, corn, lettuce and cabbage are the most widely cultivated products. Fruit is also an important product in the area, with a vast production of grapes, and 30 other species of fruits. To a minor extent, potatoes, lentils, beans, and some cereals are also cultivated, mainly for local consumption.

FIGURE 5.1: LOCALITY OF LIMACHE

FIGURE 5.2: RURAL SURROUNDINGS OF LIMACHE

FIGURE 5.3: A VIEW OF A STREET IN LIMACHE



5.2 Selection of Subjects

This thesis was part of a non-concurrent prospective study aimed to assess risk factors in early childhood and in adult life of asthma and cardiovascular disease in Chilean young adults. The fieldwork was carried out between January 2001 and April 2003.

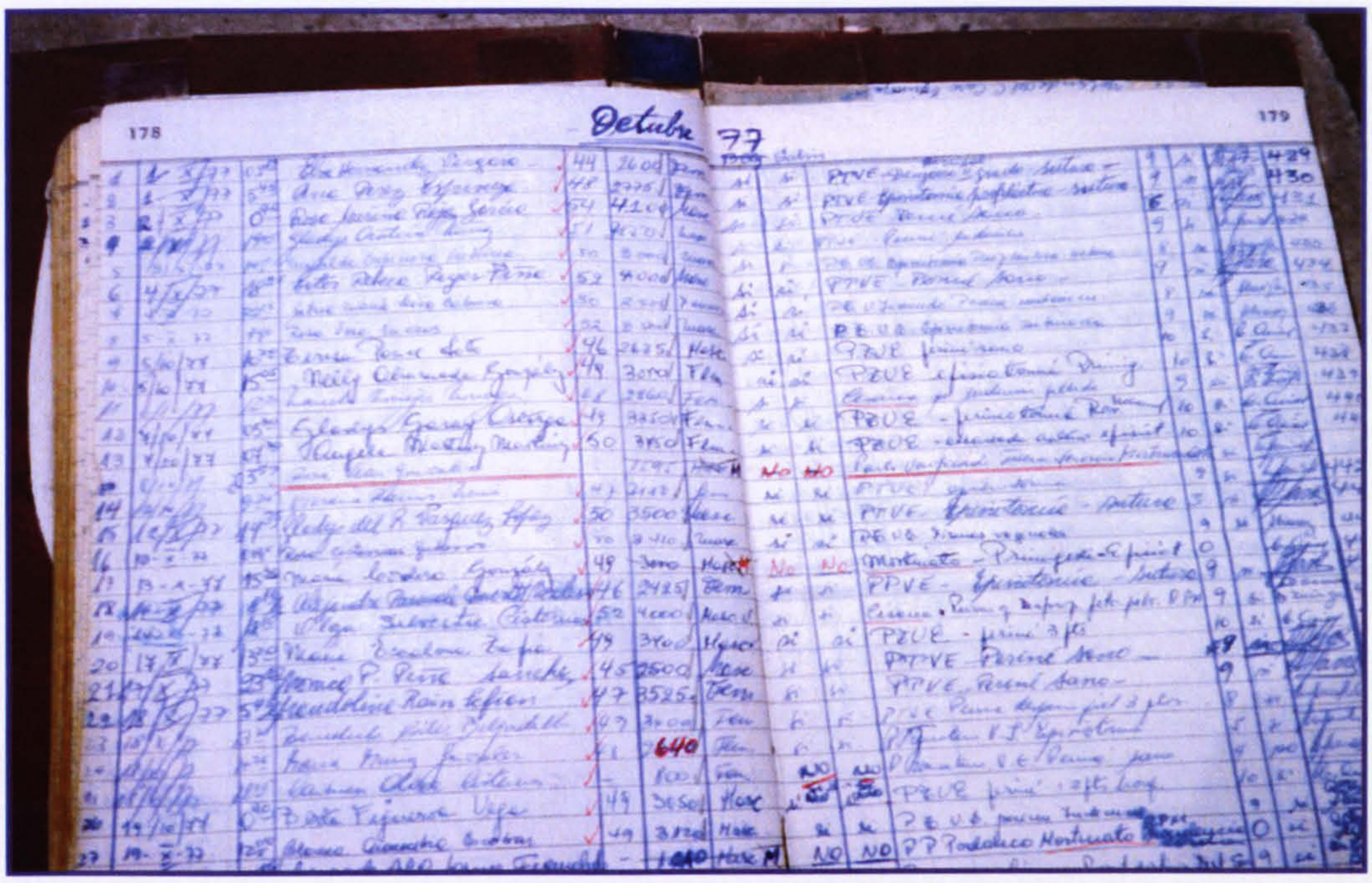
A sampling frame of 3,096 individuals, corresponding to all the births that took place in the Hospital of Limache and in Olmue between 1974 and 1978, was used to randomly select 1,232 adults that were involved in the main study. There were a number of individuals that were prevented from taking part in the study and thus randomly replaced until reaching the required sample number. Main excluding reasons consisted of emigration from the area (11.3%), unwillingness to participate (7%), death (3.2%), and custodial sentence, disability, or lactation (3.3%).

This locality and its rural surroundings were chosen because of the existence of obstetric and paediatric records for all births since 1966. The rate of emigration to other parts of the country is below 30% per decade. Health assessments were carried out in one location and most of the major socio-economic groups of the country were represented. Even in cases when a participant may have left Limache it was envisaged that their parents or other close relatives would be available and through them it would be possible to contact the subject selected in the sample.

In those years, births were recorded manually in books kept in the hospital. An example of this is provided in Figure 5.4, with a page of the book corresponding to the registry of births of October 1977. The left hand side page indicates from left to right, the date of birth, time, full name of the baby, length, weight, and whether a female or a male. The right hand side page provides additional information on the type of delivery the mother had, and the signature of the person who filled in the pages (normally a midwife or midwife assistant)

The study was approved by the Ethics Committee of the Faculty of Medicine, University of Chile.

FIGURE 5.4: A BOOK OF BIRTH REGISTRIES IN THE HOSPITAL OF LIMACHE



5.3 COLLECTION OF DATA FOR OUTCOMES STUDIED

All the assessments in this study were carried out by university nurses specially trained for this survey. Participants were invited to attend a morning session in a room of the Hospital of Limache, specially adapted for the study. Contact with selected participants was mainly made through telephone when possible, or they were approached personally. An example of the visits carried out by the fieldworkers at the house of one participant is shown in Figure 5.5.

The assessments included respiratory questionnaires, spirometric measurements, methacholine challenge, and dietary food frequency questionnaires.

FIGURE 5.5: AN EXAMPLE OF A HOUSE VISITED BY FIELDWORKERS

5.3.1 Respiratory Questionnaire

In order to ascertain information on asthma symptoms and related risk factors, a Spanish translation of the Main Questionnaire of the European Respiratory Health Survey (ECRHS) was used [294]. This questionnaire was designed and adapted from that of the International Union Against Lung Tuberculosis Diseases (IUALTD) as part of a study aimed to obtain a standardized assessment of asthma prevalence and some of its risk factors in adults from Europe. It includes questions on respiratory symptoms and medical history; occupation; information on damp, mould, soft furnishing and exposure to domestic gas appliances; air pollution; use of inhaled and oral drugs for the treatment of breathing problems; and use of services. The validity of the ECRHS questionnaire has been assessed [295].

Most of the information requested in the ECRHS Main Questionnaire was used in the Chilean study, with a few modifications to make it appropriate for the population studied (Table 5.1). The questions to ascertain respiratory symptoms in the Chilean study were the same than those of the ECRHS (Appendix 1 A). Although the question of whether asthma was diagnosed by a doctor was asked to the participants,

this was not considered as a main outcome. The rationale for this was that very few people in Limache would actually have their asthma diagnosed by a physician, found to be 38 in the sample.

Table 5.1: Differences in type of questions included in questionnaires of the Chilean study and that of the ECRHS

Item	ECRHS	Chile
On cough and phlegm	No further question on the cause of attack of cough	Did this attack of cough have coincided with having a cold?
On asthma	Are you currently taking any medicines including inhalers, aerosols or tablets for asthma?	Has a doctor given you written instructions on how to manage your asthma, or what to do in case of an asthma attack?
On asthma (women only)	Have you ever noticed that your asthma got worse with your monthly cycle? Have you been pregnant since your asthma started? What happened to your asthma during your pregnancies?	Not included
On allergies	Have you ever had an itchy rash that was coming and going for at least 6 months?	Have you ever had an itchy rash that was coming and going for the last 12 months?
On management of asthma	Several questions regarding medical attention required as consequence of asthma, including GP, nurses, and physiotherapist. Likewise it is asked if the patient has had any lab tests to check his/her condition of asthmatic. A number of questions are asked regarding the type of inhaler an asthmatic patient is using, including specific oral drugs (i.e. beta-2-agonists, methylxanthines, steroids, anti-leukotrienes, ketotifen)	An item was included about any of 10 types of medicines used to alleviate respiratory symptoms and if yes, the form of use (inhaled, tablets or syrup), the daily frequency and the last month he/she used them.

5.3.2 Spirometric measurements

All the respiratory manoeuvres were carried out by nurses, who explained in detail what the test consisted of to the participants. The nurses stated that the aim of the test was to find out how much air could be blown out of the lungs and how forcefully it

can be blown out. In Appendix 1 B, the Lung Function Questionnaire (Part 1) and Protocol of Data Collection Sheet (Part 2) are included. FEV_1 and FVC were measured using a Vitalograph device model 2120 Spirotrac IV. These measurements were performed according to the recommendations from the American Thoracic Association (1987) because in Chile reference values have been published using these recommendations [296]. FEV_1 as percentage of predicted value was based on Knudson and colleagues' recommendations [297], which were used to calculate the estimations in the Vitalograph.

With the participant in standing position and with a nose clip, he/she was asked to follow these steps: take in as much breath as possible; place the mouthpiece in his/her mouth; close his/her lips tightly around the mouthpiece; and finally, blow through the mouthpiece into the spirometer, blowing the air out as hard, fast and fully as possible. The participant was asked to continue pushing air out for as long as possible to obtain the FVC value, or to stop when the nurse said so, to obtain the FEV_1 value. Throughout this procedure, the nurse was providing positive encouragement and confidence to the participant so that he/she could push out as much air as possible.

If he/she failed to produce at least two technically satisfactory manoeuvres after five attempts, the nurse carefully explained again how to conduct the manoeuvre and allowed four more attempts. Any participant who was unable to produce two technically satisfactory manoeuvres after nine attempts was not further required to do the procedure and no FEV_1 or FVC data were collected for that individual. The number of attempts rejected was recorded in the Lung Function Data Collection Sheet (Appendix 1B, Part 2).

An unsatisfactory manoeuvre was considered as such if there was (a) a start of expiration with excessive hesitation or false start, (b) coughing during the first second of the manoeuvre, thereby affecting the measured FEV_1 value, or any cough that interfered with the accurate measurement of FVC; (c) a leak in the system or around the mouthpiece; (d) an obstructed mouthpiece. The highest value for FEV_1, (best FEV_1) produced in up to five satisfactory measures was used in the analysis.

5.3.3 Methacholine challenge

The tidal breathing method with varying concentrations of methacholine was used to assess bronchial responsiveness to methacholine challenge [298]. This modality of challenge was chosen because the challenge with concentration is the most commonly used method in Chilean hospitals. The nurses that worked in this study were trained in a hospital in the capital, specialized in the technique of challenge with concentration of methacholine.

The participants were advised to avoid smoking for 1 hour, using a β_2-agonist or anticholinergic inhaler for 4 hours or oral medication (β_2-agonist, theophylline or antimuscarinic) for 8 hours before the test. Those reporting a heart condition, epilepsy, current pregnancy or breastfeeding were excluded.

After the baseline spirometry was carried out, the nurses recorded the best initial FEV_1 as a percentage of the total predicted (Appendix 1B, Part 2). The two minutes tidal volume breathing protocol was used for those whose FEV_1 was at least 70% of predicted value. A Hudson nebuliser was used, which delivered a flow of 0.13 ml/min of saline diluent while the subject was breathing at tidal volume. A Vitalograph 2121 and software Spirotrac IV following the ATS norms [299] was used for FEV_1 measurements.

FEV_1 was measured about one minute after the nebulisation and best control FEV_1 as a percentage of the best initial FEV_1 calculated. If this value was less than 90% of the best initial FEV_1, the methacholine challenge was not carried out and administering 40µg salbutamol was used to reverse bronchoconstriction. Increasing concentrations of 0.5, 1.00, 4.00, 8.00 and 16 mg/ml were subsequently administered and FEV_1 was measured after each tidal breathing period. The test ended when a ≥20% fall of FEV_1 occurred or a concentration of 16 mg/ml was reached.

5.4 ASSESSMENT OF INDEPENDENT VARIABLES

5.4.1 Assessment of dietary intake

5.4.1.1 Characteristics of the Food frequency questionnaire (FFQ) and its administration to the participants

The consumption of 65 food items was assessed through the use of a semi-quantitative FFQ assessing dietary intake over the last month. The FFQ included a range of staple foods, antioxidant-rich foods and fatty foods (rich in saturated and hydrogenated fatty acids). The foods included in the FFQ represented approximately 90% of common daily intake of food in the Chilean population [301]. The selection criteria also considered whether these foods were part of the most usual diet of the population.

Appendix 1 C shows the FFQ used for this study. The questionnaire included 9 major groups of foods: (1) fruits with high levels of vitamin C and flavonoids; (2) vegetables rich in vitamin A and E; (3) legumes; (4) animal meat (chicken, beef, poultry, pork); fish with high levels of fat (salmon, tuna) and shellfish. Eggs were also included in this group for their high content of animal proteins; (5) cereals (bread, pasta, rice, sweet biscuits and cakes); (6) fatty foods (offal, margarine, butter, bacon, sausage and frankfurter); (7) dairy foods (whole or skimmed milk, cheese, yoghurt, milky desserts); (8) sugar, jam, honey, sweets; (9) others: red wine; non-alcoholic drinks such as juices, tea, and coffee. An additional question was added on consumption of nutritional supplements.

The participants were asked the amount and frequency of consumption of each food. Subjects were asked as follow: *"How often do you eat, e.g., oranges in a week?"* If the person replied that they ate that food item less frequently, they were asked "how often?" The second column of the FFQ registered the response of the participant, as to how many times per week/month they ate each item. In the case of bread, tea, coffee, sugar, and salt, the person was asked *"How often do you eat, e.g., bread in a day?"* The rationale for this was that these foods are part of the daily diet of the Chileans, and their consumption is very frequent.

Secondly, the participants were asked *"When you eat this item, how much do you normally eat?"* The fieldworkers showed them samples of home-size portions to facilitate the identification of the size of the portion consumed by the person. The information was registered in the third column.

Afterwards, the fieldworker had to fill in column 4 of the questionnaire, transforming the home-size portions into grams multiplying by the frequency of consumption. The equivalent standards in grams corresponding to the home-portions were taken from the guidelines of the Chilean Ministry of Health [302]. These guidelines are intended to be a reference of what should be eaten on a daily basis to keep health and to avoid chronic diseases related to an excessive intake of fats and sugars. They give a list of the foods according to the recommended intake, specifying those amounts in portions that are easily understood by the general population (tea spoon, cup, units, etc).

The last step was to obtain an estimated daily consumption of each food item in grams. For this, the fieldworker divided the grams obtained in column 4 by 7 or 30, depending on whether the subject reported consuming that item weekly or monthly, respectively. An example of this calculation is provided in the FFQ included in Appendix 1 C.

Nearly all the food items included in this FFQ were available for consumption throughout the year. An exception was the intake of strawberries, which are only harvested in summer and in this area it is very uncommon for them to be available other times of the year. The fieldwork was carried out over two years and therefore some people were interviewed in summer, but for those who were not, they were specifically asked *"When you eat strawberries in summer time, how often and how much do you normally eat?"* The procedure to fill in the rest of the FFQ was the same as that followed with the other food items.

5.4.1.2 Validation of FFQ

In order to evaluate the validity of the questionnaire used in this study, the FFQ was compared against a 24 hours-recall questionnaire used as the standard. A sample of 40 participants responded to the FFQ followed by three non-consecutive 24 hours

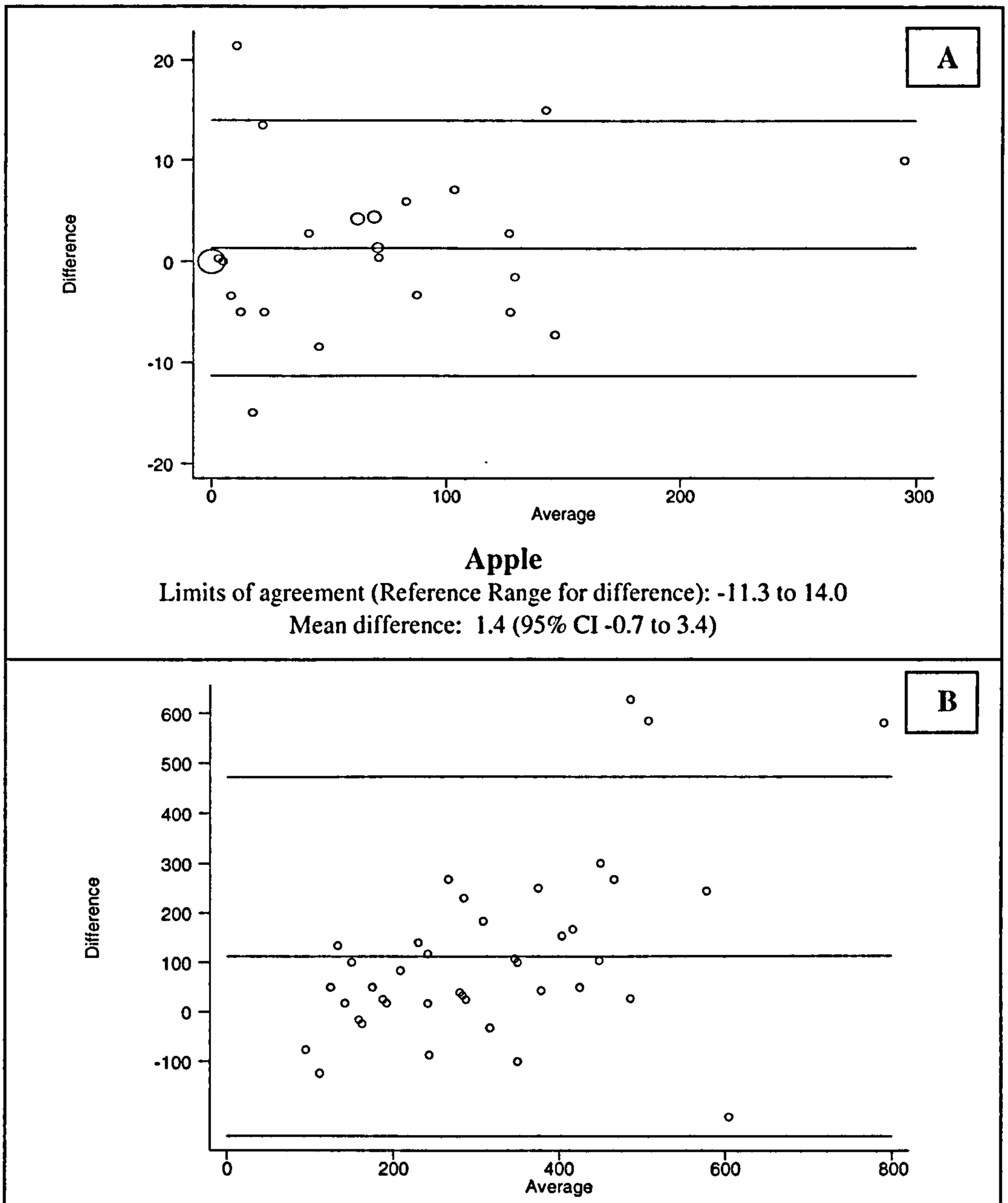
recalls, administered so as to obtain information on dietary intake during weekdays only. Participants were asked to describe all those foods and meals that they had had the previous day. Food intake was recorded as 'standard portion-sizes' and then translated into grams. Only those foods common to both questionnaires were analysed (21 items). Although fish was of interest for its content of omega 3 fatty acids, it was omitted from the comparison due to the lack of consumption reported in the 24-hours recall.

Mean intake of each food estimated by the FFQ was compared with that obtained from the 24-hours recall. Limits of agreement and mean difference between methods were calculated as recommended by Bland and Altman [303]. The differences between the measures of each food item by participant were plotted against the average of the two to provide a visual assessment of the variation of differences. The intra-class correlation coefficient (ICC) was also calculated as a relative measurement of agreement.

Compared with 24-hour recall questionnaire, the FFQ overestimated energy intake (347.3 kcal/day), as well as consumption of citric fruits, beef, bread, potatoes and avocado, but underestimated consumption for four vegetables. With few exceptions, the ICC for fruits, vegetables, dairy products and beverages was 0.9 or above, denoting high level of agreement confirmed by reasonably narrow limits of agreement. The limits of agreement for orange and avocado were wide with an ICC of 0.47 and 0.58, respectively. The ICCs for cereals were mediocre (between 0.23 and 0.48) and poor for pulses and meats, consistent with the wide limits of agreement for these food items.

Two examples are presented in Figure 5.6. In Figure 5.6 (A) the graph shows that consumption of apple had a relatively good agreement. Both in the FFQ and the 24-hours recall its intake was reported with a similar frequency. This is observed by the narrow limits of agreement and the mean difference close to zero. The case of bread (Figure 5.6 (B)) shows that its consumption was overestimated in the FFQ compared to the 24-hours recall with a large difference of means and wide limits of agreement. Overall, it was concluded that the FFQ appeared to be a good instrument to assess usual intake of foods rich in antioxidants.

FIGURE 5.6 EXAMPLES OF BLAND AND ALTMAN PLOTS FOR AGREEMENT IN VALIDATION OF FFQ AGAINST A 24 HOURS DIETARY QUESTIONNAIRE



Apple

Limits of agreement (Reference Range for difference): -11.3 to 14.0

Mean difference: 1.4 (95% CI -0.7 to 3.4)

Bread

Limits of agreement (Reference Range for difference): -248.7 to 471.5

Mean difference: 111.4 (95% CI 53.8 to 169.0)

5.4.2 Nutrient estimates

The American Table of Food Composition is the reference used in Chile for estimates of nutrient intake and therefore was used in our study for the determination of nutrients [304].

The program for nutritional analysis was based on the Nutrient Database of the United States Department of Agriculture (USDA), which provides information on energy and 28 nutritional components in more than 5,000 foods [305]. The surveys that contributed to this database were the Nationwide Food Consumption Survey and the Continuing Survey of Food Intakes by Individuals conducted by USDA, and the National Health and Nutrition Examination Survey conducted by the Department of Health and Human Services. This nutrient database has been widely used in dietary surveys in Chile [306-308]. Nutritional information provided by the Chilean Chemical Food Composition Table (which gives a description of macro and micro-minerals for a wide range of staple food produced or harvested in the country) was also used to obtain chemical composition of staple foods produced locally (e.g. kneaded yeast bread) [309].

In order to assess the levels of agreement and comparability of estimates obtained from these sources of food composition, in collaboration with my supervisors I carried out a comparison against the British Table of Food Composition [310]. The nutrient estimates of the British Table of Food Composition were obtained using the program IDA (Integrated Dietary Analysis) [311], based on the Royal Society of Chemistry Database 1988-1995, plus all available supplements and appendices, obtained from a series of analytical studies commissioned by the Ministry of Agriculture, Fisheries and Food [312, 313].

Estimates of nutrients using each of the two sets of tables of food composition were independently obtained, in Chile for the USA (Chilean) Table of Food Composition and in the UK for the British Table of Food Composition. Although the British Tables are not adapted to the peculiarities of the Chilean diet this did not constitute a major problem, as only one item of food, 'longaniza', was unavailable and finally entered as 'sausage'. Agreement between estimates was expressed by limits of agreement, as recommended by Bland and Altman [303].

The mean differences between the two tables of food composition showed higher estimates when using the American (Chilean) Table for macronutrients (that ranged from 5.3% to 8.9%). For micronutrients, a bias towards a higher mean was observed for vitamin E, iron and magnesium when the American (Chilean) Table was used,

but the opposite was observed for vitamin A and selenium. The ICC ranged from 0.86 (95% CI 0.81-0.91) for iron to 0.998 (95% CI 0.995-1.00) for vitamin A, indicating high to excellent agreement. Limits of agreement for macronutrients and vitamins A and C were satisfactory.

5.4.3 Flavonoid data

As in Chile there are no local data available on the flavonoid content of foods, this intake was established using Dutch food composition data compiled between 1992 and 2000 [314-317], which were based on analyses by high performance liquid chromatography, of a comprehensive set of commonly consumed foods, taking on board seasonal and year-to-year variability.

Three major classes of flavonoids were obtained: flavonols (quercetin, kaempferol, myricetin), flavones (apigenin, luteolin) and catechins [(+)-catechin, (+)-gallocatechin, (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin gallate, and (-)-epigallocatechin gallate]. The symbols (+) and (-) refer to the molecular structure of the different catechins. These flavonoids are chemically known as enantiomers. This means that each of the catechins has a unique spatial configuration that makes them different in molecular structure to the other catechins [318].

5.4.4 Biomarkers

Four biomarkers were assessed in plasma: antioxidant capacity of plasma, uric acid (both biomarkers of antioxidant status), F2-isoprostanes and protein carbonyls (biomarkers of oxidative stress). The techniques for assessment of these biomarkers were developed in the Laboratory of Pathophysiology, part of the Faculty of Medicine, University of Chile. Blood samples were collected in the morning (30 mL) and stored at -68 degrees Celsius until they were analysed. Data were collected for 600 participants because the sample collection for this purpose started later than the rest of the data collection.

5.4.4.1 Antioxidant capacity of plasma (FRAP assay)

FRAP was calculated according to the method of Benzie and Strain. The technique uses a ferric-tripyridyltriazine complex that is reduced to the ferrous (Fe^2) form. The reagents were obtained from Hoffman-LaRoche Ltd. Switzerland. The technique is a good mirror of the effect that vitamin C, E and flavonoids have in plasma, as it captures their scavenging properties [262].

5.4.4.2 Uric acid in plasma

Uric acid was assessed through an enzymatic method [319] *Human GMbH* provided the reagents. Plasma uric acid is a potent antioxidant capable of reducing some free radicals through the donation of electrons under physiological or pathological conditions. Jointly with vitamin C it is considered one of the most powerful scavengers in plasma.

5.4.4.3 Isoprostanes (F2-ip)

Levels of F2-ip were assessed in plasma by immunoassay test ELISA [320]. The KIT of 8-isoprostane EIA was provided by CAYMAN Chemical Company. The technique used measures the free amount of 8-epi-prostaglandin $F_{2\alpha}$ that it is produced as consequence of the oxidation and release of arachidonic acid to the extra-cellular environment.

5.4.4.4 Protein carbonyls

Carbonyls of proteins were assessed by the method of Reznik and Packer, using a spectro-photometric method [321]. The method measured the reaction of di-nitro-phenyl-hydrazine with protein carbonyls to form protein hydrazones, and results were expressed as nmol carbonyl/mg protein. This occurs as consequence of the attack of oxygen radicals to specific bonds of amino acids of the protein, shifting its structure, initiating carbonyl formation and leading to the degradation of the protein. The formation of these carbonyls indicates that oxidative stress took place.

5.5 STATISTICAL ISSUES

5.5.1 Dependent variables

Symptoms of asthma and BHR to methacholine (as slope) were the primary outcome measures in the study. Asthma symptoms were ascertained as follow: (1) wheezing in the last 12 months, (2) woken with shortness of breath, and (3) having at least one respiratory symptom (wheezing in the last 12 months, woken with shortness of breath in the last 12 months, and breathlessness at rest).

As the simple percentage of PC_{20} <16mg/ml wastes the information in the size of PC_{20} in those with an estimate, a least squares concentration-response slope was used, analogous to dose-response slope [322]. The slope was calculated with a regression of the percentage fall of FEV_1 on concentration for each participant.

For the statistical analyses, lung function was analysed as the highest (best) value of FEV_1 reached out of five attempts, and the FEV_1/FVC.

5.5.2 Independent variables

Five groups of explanatory variables were included as independent variables and analysed as quintile groups. These were: (1) fruits, (2) vegetables, (3) nutrients, (4) flavonoids, and (5) biomarkers of antioxidant status and oxidative stress. Fruits and vegetables rich in antioxidants and vitamins were grouped and their total intake analysed. Total daily intakes of orange, kiwi, strawberry, and mandarin were summed and defined as "total fruit intake". For "total vegetable intake", daily total intake of the following food items was considered: beetroot, chard, sweet pepper, garlic, onion, tomato, potato, pumpkin, carrot, and avocado. Secondary analyses were also carried out individually with these and other food items of potential interest for their relation with the main antioxidant hypothesis being tested or for their association with asthma. A number of food items rich in saturated and mono unsaturated fatty acids were also included (Appendices 2 and 3).

Antioxidant nutrients with described antioxidant capacity in relation to asthma and lung function were included in the main analyses: vitamins C, E and total vitamin A; minerals selenium and zinc. Additionally, intake of omega 3 fatty acids and ratio n6/n3 was also analysed for their role in the antioxidant/oxidant balance in the respiratory system.

Three major subclasses of flavonoids were analysed: flavonols, flavones, and catechins. As quercetin (the main flavonol) was very highly correlated with total flavonol intake, flavonols were analysed as a group only. Similarly, as intake of epicatechin was highly correlated with catechins and total catechins, the three main contributors to the intake of total catechins in this study, the analyses are presented as total catechins.

Levels of biomarkers in plasma were also included as explanatory variables: FRAP, uric acid, F2-ip and carbonyls of proteins. Correlations between biomarkers with each other and with food items were calculated.

5.5.3 Potential confounders

Adjustments were made for sex, age, height (for FEV_1 only), current smoking, overcrowding, number of years of education, socio-economic status, weight at birth, BMI (weight (kg)/height $(m)^2$), and TEI.

For the current study, the associations between BHR slope and explanatory variables were analysed considering the additional potential confounders FEV_1 percentage predicted, FEV_1%FVC, and atopy as it has been demonstrated in general population that these variables are independently related to BHR [323].

Skin test reactivity to eight allergens (supplied by Allergy Therapeutics) was performed: *D pteronyssinus*, cat fur, dog hair, *Alternaria alternata*, cockroach, a mixture of grass pollens considered the main contributors to pollen in the air in Santiago [300] (oats grass, crested dogstail, cocksfoot, rye grass, meadow grass, vernal bent, brome, meadow foxtail, Timothy, meadow fescue, Yorkshire fog), a mixture of weeds and shrubs (mugwort, fat hen, orache, nettle, plantain), and a

mixture of trees (birch, beech, oak-common, alder, ash, hazel, poplar, plane, elm, willow). Histamine was used as the positive control and an uncoated Phazet as the negative control.

The tests were performed on the volar surface of the forearm using a standard template and the wheal size was recorded at 15 minutes as the biggest diameter and the diameter at 90° to its midpoint, each to the nearest whole millimetre. The mean wheal of the diameters was then calculated. A skin prick reaction was regarded as positive if the wheal mean diameter was 3mm or greater. Subjects were considered atopic if they have a positive reaction to any of the allergens tested.

The criterion for defining socio-economic status in this population was based on previous analyses on the association between multiple socio-economic indicators with asthma in the adults taking part in the main study of Limache [324]. These indicators were investigated taking into account that this population was comprised of young adults aged 22 to 28 years, and in Chile it is common that they are not fully independent from their parents at this stage. The measures asked in the questionnaire included years of primary, secondary or higher education in the participants and their parent, breadwinner occupation, an index of type and ownership of house as some people may have owned a poor quality house, while others rented/owned a solid one; household overcrowding; number of belongings reflecting socio-economic status such as gas-fuelled water heater device, personal computer, fridge, washing machine, and microwave oven; car ownership; completion of a welfare form and total number of siblings.

Previous analyses showed a statistically significant association between having fewer 'number of belongings' (0 to 2 compared to 3 to 5) and each of the asthma symptoms under consideration: wheezing in the last 12 months (OR 1.39, 95% CI 1.01 to 1.80; p=0.01), wheezing and another asthma symptom such as waking up with breathlessness or breathlessness at rest (OR 1.65, 95% CI 1.20 to 2.40; p=0.007), and frequent wheeze or another asthma symptom (OR 2.07, 95% CI 1.10 to 4.04; p=0.04) [324].

5.5.4 Sample size calculation of the main study and detectable difference for 90% power in this thesis

The sample size for the main study was calculated based on the regression coefficient of FEV_1 on birth weight in a previous study [325] and converted into a correlation. It considered birth weight because of the programming and early life risk factors hypothesis being tested. As stated in the application to the Wellcome Trust Fund: "*With a sample of 1,100 subjects, it was possible to detect at the 5% level with 80% power, a correlation of 0.085 (corresponding to a regression coefficient of 0.624 SDs/Ratio birthweigth/gestational age) between FEV_1 and birth weight*". A sampling frame of 3,096 individuals, corresponding to all the births that took place in the Hospital of Limache between 1974 and 1978 was used to randomly select 1,300 to allow for losses. A total of 1,232 were involved in the main study. A number of individuals were prevented from taking part in the study and thus randomly replaced.

The determination of detectable difference in the current study was carried out post-hoc. The SDs used were for intake of vitamins C and E, and selenium reported by asthmatics and non-asthmatics in the study of Picado and colleagues [112] that included 118 asthmatic subjects aged 41.6 (SD 1.4) and 121 controls aged 38.8 (SD 1.3). The aim of that study was to assess the possible relation between dietary intake of macro and micronutrients and asthma. Three criteria were used to define asthma: having wheeze in the last 12 months; having two or more of the following respiratory symptoms: wheezing, waking up with shortness of breath, and day time shortness of breath at rest; and answering 'yes' to the question of having asthma (Table 5.2).

Taking as example vitamin E and the 327 cases who reported having wheeze in this population, this study has 90% power to detect a difference between the means of intake of vitamin E between 6.25 and 6.7 assuming that the SD in the asthmatic subjects is 2 and the SD in the non-asthmatics is 2.4 [112] (Table 5.2).

Table 5.2: Determination of detectable difference in nutrient intake with 90% power at the 5% level of significance in asthmatic (A) and non-asthmatic (NA) adults

Criterion	Detectable difference in milligrams (SD used in calculation)					
	Vitamin E		Vitamin C		Selenium	
	NA	A	NA	A	NA	A
According to those who had wheezing in the last 12 months (n=327)	6.7 (2.4)	6.25 (2.0)	165.0 (98.0)	147.5 (75.0)	78.0 (35.0)	72.5 (20.0)
According to those participants with two or more of the following respiratory symptoms: wheezing, waking up with shortness of breath, and day time shortness of breath at rest (n=186)	6.7 (2.4)	6.15 (2.0)	165.0 (98.0)	144.5 (75.0)	78.0 (35.0)	72.0 (20.0)
According to those who answered 'yes' to whether they were asthmatics (n=54).	6.7 (2.4)	5.75 (2.0)	165.0 (98.0)	130.5 (75.0)	78.0 (35.0)	68.5 (20.0)

5.5.5 Analyses

All independent variables were tested for linearity in relation to each of the outcomes included in this thesis. As there was no evidence for non-linearity, the explanatory variables are presented as quintile groups with P-values for linear trend.

Multiple linear regressions and multiple logistic regressions were used to assess the association between the outcomes and risk factors. Multiple linear regression analyses were used to assess the association between measurement of FEV_1, FEV_1/FVC and BHR, with fruit, vegetable, nutrient and flavonoid intake. The adjusted model included as potential confounders sex, age, height (in the case of FEV_1), socio-economic variables (grouped as explained above), weight at birth, BMI, and TEI. For BHR slope the potential confounders FEV_1%, FEV_1%FVC and atopy were also added [323]. The association between respiratory symptoms of asthma and the independent variables was examined with multiple logistic

regression, including the following potential confounders: sex, age, socio-economic variables, weight at birth, BMI, and TEI.

To examine the association between each of the outcomes studied and biomarkers as independent variables, TEI was not included in the multiple regressions. The rationale for this is that TEI is unlikely to affect levels of biomarkers of oxidative stress or antioxidant status included in the current study.

As a large number of comparisons were carried out, adjusted p values were modified according to the Bonferroni method [326]. P-values were multiplied by the number of tests carried out for each outcome i.e. in each table. This approach was chosen due to the outcomes being correlated. Corrected P-values are shown only when the nominal P-value was less than 0.05.

The Bonferroni correction was also applied in the results shown in appendices 2 and 3, for the analyses that shown a nominal P-value less than 0.05. In those cases where the Bonferroni P-value was higher than 1.0 is stated to be 1.0 [326].

The statistical analyses were performed with STATA 8.2 [327].

CHAPTER 6

RESULTS I: Characteristics of the population and their dietary intake

6.1 GENERAL CHARACTERISTICS OF THE POPULATION

1192 out of 1232 adults in the study sample completed the FFQ. The exclusion of 40 cases occurred because this study started shortly after the main one and it was not possible to contact again these 40 individuals to obtain dietary information. The sample included a slightly greater proportion of women (54.4%). Women were more likely to stay at home and look after the family while the men were more likely to be working in the fields or in town, being more difficult for them to enter the study, which took place during weekdays.

The median BMI was near to the overweight cut-off in men, and just over 25 in women, indicating that around 50% of the participants were overweight (Table 6.1). The mean height of men and women was considerably lower than the mean of height in western developed countries.

Table 6.1: General characteristics of the participants

Characteristic	Males (n=544)	Females (n=648)
	Mean (SD)*	Mean (SD)*
Age (yr)	24.9 (1.6)	24.7 (1.6)
Adult weight (kg) Median (IQR)	70.1 (63.0 to 77.9)	61.5 (54.9 to 70.0)
Adult height (cm)	168.1 (6.1)	156.3 (5.5)
BMI (kg/m^2) Median (IQR)	24.7 (22.6 to 27.2)	25.1 (22.6 to 28.6)
Weight at birth (g)	3184 (488.2)	3192 (508.0)
Length at birth (cm)	49.4 (2.2) (n=542)	49.4 (2.1) (n=647)

* Except as stated

Information on lung function was obtained in 1,187 cases. Men had a mean FEV_1 1L higher than women. Wheeze in the last 12 months was reported by over a quarter of the participants. Women reported higher prevalence of waking with shortness of

breath and breathlessness at rest and 45% had at least one respiratory symptom (Table 6.2).

Table 6.2: Characteristics of dependent variables and some confounders included in the study

	Variable	Males	Females
Measurements of lung function			
Lung Function		N Mean (SD)	N Mean (SD)
	FEV_1 (l) ^	542 4.12 (0.53)	645 3.09 (0.38)
	FEV_1 as % of predicted value for age and sex #	541 105.2 (10.91)	645 105.3 (10.69)
	FEV_1/FVC	542 0.86 (0.047)	645 0.88 (0.048)
Prevalence of respiratory outcomes		N %	N %
Respiratory symptoms	Wheeze in the last 12 months	143 26.3	184 28.4
	Having at least one respiratory symptom (wheeze, woken with shortness of breath or breathlessness at rest)	202 37.1	291 44.8
	Woken with shortness of breath	58 10.7	107 16.5
	Breathlessness at rest	89 16.4	148 22.8
Atopy	Sensitised to at least one allergen	145 26.7	171 26.4
	Atopy and wheeze in the last 12 months	51 9.4	60 9.2
	Atopy and at least one respiratory symptom	71 13.1	78 12.0
BHR 16mg/mL	Positive response to methacholine	38 7.5	98 16.3
	BHR as slope: N, mean (SD)	504 -0.05 (1.48)	602 0.002 (1.65)
Confounders		N %	N %
Smoking	Current smoker	367 67.5	319 49.2
Education	12 years of full time education	272 50.0	324 50.0
Estimation of socio-economic level	No of household belongings * 0 or 1	75 13.8	93 14.3
	2	164 30.2	223 34.4
	3	173 31.9	211 32.5
	4 or 5	131 24.1	122 18.8

^ Highest of five measurements
# FEV_1 as percentage of predicted value was calculated from Knudson's recommendations [297]
* Having a washing machine, microwave, gas-fuelled water-heating device, refrigerator or a computer at home

A quarter of the participants were sensitised to at least one allergen. The combination of atopy and at least one respiratory symptom was present in just over 10% of the participants. BHR prevalence, defined as a 20% fall in FEV_1 to a concentration of methacholine of up to 16 mg/mL, was twice as common in women as in men.

A high percentage of the sample population were current smokers. Full high school education was accomplished by 50% of the participants.

In the current study, there were no participants reporting use of any type of asthma medication

Plasma levels of FRAP, uric acid, and protein carbonyls were calculated for 585 cases. 16 samples were not included in the analyses as the blood samples arrived defrosted to the laboratory in Santiago. In the case of F2-ip, 564 samples were included in the analyses. As before, some samples arrived unfrozen to Santiago (n=27), and in 9 cases the laboratory technique failed and it was not possible to carry out the determination of the biomarker again due to insufficient blood. Levels of FRAP and uric acid were 20% average higher in men than in women, while levels of F2-ip were 12% greater in males. Levels of protein carbonyls were very similar in the two groups (Table 6.3).

Table 6.3: Basal levels of biomarkers

Biomarker	Males		Females	
	N	Median [IQR]	N	Median [IQR]
FRAP (μM)	284	340.3 [291.8 - 407.8]	301	285.5 [243.5 – 333.7]
Uric Acid (mg/dL)	284	4.7 [4.0 -5.6]	301	3.9 [3.3 – 4.7]
Protein carbonyls (nmol/mg protein)	284	0.9 [0.6 – 1.1]	301	0.8 [0.6 – 1.1]
F2-ip (pg/mL)	277	28.0 [22.5 – 36.1]	287	24.9 [19.1 – 33.7]

The two biomarkers of antioxidant status FRAP and uric acid showed the highest correlation among all the biomarkers. FRAP was also positively and statistically significantly correlated with carbonyls and F2-ip, although more weakly than that observed with uric acid. A positive correlation was observed between carbonyls and F2-ip, which reached statistical significance (Table 6.4).

Table 6.4: Pearson's correlation between biomarkers

	Correlation [95% confidence interval] (p value)		
Biomarker	**FRAP**	**Uric acid**	**Carbonyls**
Uric Acid	0.29 [0.22 to 0.37] (<0.001)		
Carbonyls	0.14 [0.06 to 0.22] (0.001)	0.04 [-0.04 to 0.12] (0.36)	
F2-ip	0.13 [0.04 to 0.21] (0.03)	0.14 [0.05 to 0.22] (0.001)	0.13 [0.05 to 0.21] (0.001)

6.2 FOOD AND NUTRIENT INTAKE

Mean consumption of fruits and fresh vegetables was slightly above the recommendations given by the Chilean Ministry of Health with its Guidelines on the Pyramid of Foods [301]. In Britain, this is comparable to the Department of Health Recommendations '5 a day', where 5 portions of fruits or vegetables are being recommended to the public as an optimum intake to prevent obesity and chronic diseases [328]. The Chilean population studied had a consumption of almost 6 units of fruits and vegetables, which may be equivalent to 7 to 8 portions of the British food guidelines (Table 6.5).

Table 6.5: Daily intake of fruits and vegetables and level of adequacy according to the Chilean Pyramid of food

Food group	**Food item**	**Mean (g) [95% CI]**	**Estimated daily home-size portion**	**Intake as recommended in the Pyramid**
Fruits	Orange	119.8 [106.9 to 132.7]		
	Lemon	22.8 [20.9 to 24.6]		
	Kiwi	34.0 [25.2 to 42.8]		
	Apple	82.5 [74.6 to 90.4]		
	Strawberry	15.8 [12.8 to 18.9]		
	Mandarin	73.4 [64.3 to 82.5]		
Total		**348.3 [325.6 to 371.1]**	3.5 units	3 units/day
Vegetables	Beetroot	22.0 [19.5 to 24.5]		
	Chard	15.2 [13.4 to 17.0]		
	Sweet pepper	8.5 [7.2 to 9.7]		
	Garlic	4.8 [4.4 to 5.2]		
	Onion	50.3 [46.6 to 54.1]		
	Tomato	183.2 [168.1 to 198.1]		
	Pumpkin	21.7 [20.6 to 22.8]		
	Carrot	40.0 [37.1 to 43.0]		
	Avocado	97.0 [87.3 to 106.7]		
Total		**442.7 [420.0 to 465.3]**	2 ¼ regular plates	2 regular plates/day

The predominant fruits consumed were orange, mandarin and apple. Tomato and avocado were the main vegetables consumed by the population, with almost two and one unit per day, respectively (Table 6.5). Estimated mean daily intake of legumes was sufficient to reach the recommended intake per week (Table 6.6).

Bread is a staple food in Chile, and thus widely consumed by the participants, with more than 3 units per day on average (371g/d), kneaded yeast bread being the commonest type of bread consumed. Intake of the rest of the cereals, rice, pasta, and potato (included in this category for its content of complex carbohydrates) were within the recommended intake (Table 6.6). Consumption of chicken and beef was roughly equivalent to a portion of each per week, which exceeds slightly the recommended intake of one portion a week. Similarly, foods with high content of sugar were commonly consumed. This was specially reflected by the consumption of soft drinks while the intake of dairy products, in particular milk, was well below the recommended amounts per day.

Intake of tea reached an average of a cup per day in this population (Table 6.6).

Intake of energy and macronutrients is presented in Table 6.7. The estimated average requirement (EAR) established by the UK Department of Health was used to estimate the adequacy of the intake [313]. As in Chile, these recommendations are based in the guidelines of FAO/WHO/UN. It could be argued that the only difference between the British and Chilean nutritional recommendations is related to the proportions of macronutrients. In Britain, the advisable percentage of fats derived from energy intake is 35%, and that of total carbohydrates is 50%. In Chile, these values are ≤ 30 and 60% for fat and carbohydrates, respectively, which has to do with the fact that cereals, and in particular bread, are main components of the staple diet in Chileans. Therefore, the British standard is used to estimate adequacy of intake in the studied population.

Table 6.6: Daily intake of other major food items and equivalent estimated home-size portions consumed based on the recommendations of the Chilean Pyramid of Food

Food group	Food item	Mean (g) [95% CI]	Estimated daily home-size portion	Intake as recommended in the Pyramid ^
Legumes	Beans	21.6 [20.4 to 22.9]	2 portions, or 2 small plates a week	1 portion twice a week
	Lentils	15.3 [14.3 to 16.3]		
	Chickpeas	4.2 [3.6 to 4.8]		
Cereals	Total Bread	371.3 [358.8 to 383.9]	3-4 units	2-3 units/day
	Rice	42.2 [39.9 to 44.6]	1 ¼ cup	Up to 2 cups of rice or 3 regular-size potatoes or 1 small plate of pasta/day
	Pasta	25.0 [23.3 to 26.7]		
	Potato	172.2 [165.2 to 180.2]		
Total *		**240.0 [231.2 to 248.8]**		
Animal proteins	Beef	53.4 [52.2 to 56.3]	1 regular portion	1 regular portion once a week
	Chicken	16.2 [15.1 to 17.2]		
	Ribs	12.3 [11.2 to 13.8]		
	Fish	10.5 [9.6 to 11.4]		
	Egg	31.2 [29.1 to 33.3]	½ unit	1 unit twice a week
Fatty foods	Oil	14.4 [13.8 to 15.0]	3 tea-spoons	Consume in small amounts
	Bacon	0.5 [0.2 to 0.8]	¼ unit	Consume in small amounts
	Sausage	5.6 [4.9 to 6.3]		
	Frankfurter	11.7 [10.8 to 12.6]		
	Ham	17.9 [16.3 to 19.5]	¼ portion or ½ small slice	Consume in small amounts
	Offal	2.9 [1.9 to 3.8]		
	Margarine/butter	10.6 [9.7 to 11.5]	3 tea spoons	Consume in small amounts
	Mayonnaise	6.0 [5.4 to 6.6]		
Dairy	Milk	105.2 [93.4 to 117.0]	½ cup or 2 slices of cheese	3-4 cups of milk or 4 slices of cheese/ day
	Cheese	12.9 [11.5 to 14.3]		
Sweets	Sugar	27.7 [26.3 to 29.0]	3 table spoons	Consume in small amounts
	Jam	2.9 [2.0 to 3.8]		
	Cake	15.2 [13.4 to 17.0]	1 unit per week	Consume in small amounts
	Honey	1.1 [0.8 to 1.5]	Trace	Consume with moderation
	Soft drink (Coke)	324.6 [299.1 to350.2]	2 regular glasses	Consume in small amounts
	Juice	55.8 [42.6 to 68.9]		
Beverages	Tea	205 [184.1 to 225.9]	1 cup	No specific recommendation
	Coffee	12.0 [8.0 to 16.0]		
	Red wine	48.5 [36.4 to 60.6]	1/3 glass	No specific recommendation
Other	Salt	5.1 [4.8 to 5.5]	1 tea spoon	6 g or 1 tea-spoon/day Avoid adding to prepared meals

* Total bread and cereals shown separately

^ Recommendations for some food items are given as portions per day or per week

Table 6.7: Estimated daily intake of energy and macronutrients in the studied population and percentage of the British recommended values

	Males			Females		
Energy & Macronutrients	**Median [IQR]**	**EAR***	**% of EAR**	**Median [IQR]**	**EAR***	**% of EAR**
Energy (kcal)	3439.5 [2665.3- 4415.5]	2550 kcal	134.9	2279.0 [1170.7-2844.7]	1940 kcal	117.5
Proteins (g)	129.6 [98.4- 163.3]	44.4 g	292	92.1 [71.0-118.1]	36.0 g	256
Carbohydrates (g) % TEI	502.6[385.1-625.2] 58.5	50% TEI	136.0	328.8 [254.4-427.8] 57.7	50% TEI	138.0
Total lipids (g) % TEI	97.9 [71.1-137.6] 25.6	35% TEI	73.1	66.1 [48.2-91.9] 26.1	35% TEI	74.6
PUFA (g) % TEI	26.2 [18.3-36.1] 6.9	6.5% TEI	106.2	18.7 [13.3-25.5] 7.4	6.5% TEI	113.8
MUFA (g) % TEI	36.6 [25.5-55.1] 9.6	13% TEI	73.8	23.3 [16.7-35.5] 9.2	13% TEI	70.8
SFA (g) % TEI	26.1 [18.9-38.1] 6.8	11% TEI	61.8	17.1 [11.7-25.1] 6.8	11% TEI	61.8
Omega 3 (g) % TEI	0.24 [0.1-0.5] 0.06	0.2% TEI^	1.2	0.2 [0.1-0.4] 0.08	0.2% TEI^	4.0
Omega 6 (g) % TEI	11.9 [7.7 -17.0] 3.1	1.0% TEI^	310	8.5 [5.8-13.1] 3.4	1.0% TEI^	340
Ratio n6/n3	48.4 [23.7 – 107.4]	2-5:1		46.4 [23.3-108.9]	2-5:1	
Cholesterol (mg)	323.1 [217.5-458.0]	200 mg	161.5	192.7 [132.6-276.4]	130 mg	148.2

* EAR= Estimated average requirements for adults aged 19-50 years
^ The British Panel of Dietary Reference values stresses that these recommendations are the individual's minimum intake.

The participants had a TEI above the EAR per day, particularly men. Carbohydrates were the main source of energy, with nearly 60% of TEI in both groups. Total lipid intake reached 75% of the British recommended values, with MUFA and SFA intake also below the recommendations. Intake of PUFA reached the recommended values, mainly due to intake of omega 6, as intake of omega 3 was extremely low. This was also mirrored in a ratio n6/n3 of 47:1, exceeding by far the recommended range of 2-5:1 [329].

With regard to intake of micronutrients (Table 6.8), consumption of nearly all vitamins was above the Recommended Nutritional Intake (RNI). Vitamins C and E were those consumed in more quantity in relation to the RNI. For folic acid the RNI was unmet both for men and women, the latter having a consumption of just over a third of the RNI. Women also had an insufficient intake of niacin, as it only reached

nearly 65% of RNI. Intake of all minerals met the RNI in men and women, with the exception of calcium, whose consumption was nearly 50% of the RNI in women. Intake of iron, magnesium and selenium duplicated the RNI in men, while women had an intake of iron within the recommended range of RNI, and a sufficient intake of minerals.

Table 6.9 shows the distribution by quintile groups of total vegetable and fruit intake, and nutrients included in the analyses. Intake of omega 3 fatty acids was below the requirements for most participants and the n6/n3 was well above the recommended ratio. For all other nutrients and grouped foods, only participants in the first quintile had a lower intake than that recommended.

Table 6.8: Estimated daily intake of micronutrients in the studied population and level of adequacy according to the British recommended values

Nutrient group	Nutrient	Males			Females		
		Intake Median [IQR]	RNI	% of RNI	Intake Median [IQR]	RNI	% of RNI
Vitamins	Carotene (µg)	1027.0 [585.6-1707.0]	800	128.4	1107.1 [681.2-1876.8]	600	184.5
	Retinol (µg)	262.5 [140.6-431.9]	150	175.0	186.6 [100.8-324.2]	100	86.6
	Total vitamin A (µg)	1412.3 [854.4-2121.7]	700	201.8	1374.5 [891.7-2328.9]	600	229.8
	Vitamin C (mg)	133.3 [84.9-235.1]	40	333.3	138.7 [81.7-229.2]	40	346.8
	Vitamin E (mg)	19.6 [14.2-26.3]	4	490.0	15.2 [10.9-19.7]	3	506.7
	Folic Acid (µg)	116.8 [83.6-150.7]	200	58.4	71.7 [52.4-94.0]	200	35.9
	Pantotenic Acid (mg)	7.0 [5.3-8.8]	3-7	100.0	4.9 [3.7-6.5]	3-7	100.0
Minerals	Calcium (mg)	618.8 [456.2-861.0]	600	103.1	433.0 [328.6-646.3]	900	48.1
	Copper (mg)	2.2 [1.7-2.8]	2	110.0	1.5 [1.2-2.0]	1	150.0
	Iron (mg)	26.6 [20.0-33.5]	10-12	216.7	17.2 [13.6-21.9]	13-18	100.0
	Magnesium (mg)	379.0 [290.1-496.1]	150	252.7	275.1 [211.4-363.1]	150	183.4
	Selenium (µg)	163.8 [119.9-203.2]	75	218.4	100.6 [77.5-129.7]	60	167.7
	Zinc (mg)	11.8 [9.2-14.7]	7	168.6	11.8 [9.2-14.7]	5	236.0
Flavonoids (mg)	Total catechins	17.1 [7.8-64.4]	ND	--	21.2 [9.3-68.5]	ND	--
	Flavonols	26.2 [15.7-44.3]	ND	--	24.7 [12.7-41.3]	ND	--
	Flavones	0.1 [0.04-0.2]	ND	--	0.1 [0.03-0.2]	ND	--

ND= Not defined

Table 6.9: Distribution of food and nutrient intake by quintiles

Food/ nutrient	Quintile groups	Limits of intake	Recommended intake (As referenced in Tables 6.5, 6.7 and 6.8)
Total fruit intake (g)	1	0.0 - 96.4	3 units per day / 300 g
	2	97.1 - 190.7	
	3	191.0 - 300.7	
	4	303.4 - 491.7	
	5	492.4 - 4419.6	
Total vegetable intake (g)	1	82.8 - 318.5	2 regular plates / 400 g
	2	318.7 - 440.1	
	3	440.4 - 580.1	
	4	582.0 - 796.0	
	5	799.9 - 4867.0	
Vitamin C (mg)	1	14.7 - 73.3	40 mg for males and females
	2	73.4 - 113.4	
	3	113.6 - 171.3	
	4	171.4 - 264.6	
	5	264.6 - 1933.3	
Vitamin E (mg)	1	3.0 - 11.3	Males 4 mg Females 3 mg
	2	11.3 - 15.1	
	3	15.1 - 18.9	
	4	18.9 - 25.0	
	5	25.1 - 121.9	
Total vitamin A (µg)	1	73.1 - 778.8	Males 700 µg Females 600 µg
	2	779.7 - 1165.3	
	3	1165.3 - 1645.2	
	4	1646.6 - 2527.6	
	5	2541.2 - 18860.9	
Omega 3 fatty acids (g)	1	0.0 - 0.07	0.2% TEI Equivalent in grams: Males: 5.7g Females: 4.3g
	2	0.08 - 0.16	
	3	0.16 - 0.26	
	4	0.27 - 0.50	
	5	0.50 - 4.1	
Ratio omega 6/omega 3	1	0.2:1 - 19:1	2-5:1
	2	20:1 - 37:1	
	3	37:1 - 62:1	
	4	62:1 - 129:1	
	5	130:1 - 5336:1	
Selenium (µg)	1	15.3 - 83.1	Males 75 µg Females 60 µg
	2	83.1 - 108.9	
	3	109.1 - 138.8	
	4	139.1 - 184.9	
	5	184.9 - 602.4	
Zinc (mg)	1	1.8 - 6.6	Males 7 mg Females 5 mg
	2	6.6 - 8.7	
	3	8.7 - 10.6	
	4	10.6 - 13.6	
	5	13.6 - 43.9	

Table 6.10 provides information on population-based surveys in Britain and Chile, with estimates of dietary intake in adults and percentage of adequacy of EAR in the case of energy and macronutrients, or of RNI for vitamins and minerals. Dietary information in Britain was obtained from the latest British National Diet and Nutrition Survey carried out between 2000 and 2001, which examined the dietary intake of a representative sample of adults aged 19 to 64 years. A 7-day weighed-intake dietary record was used for the quantification of food and nutrient intake in 1,724 respondents [330]. The values for micronutrients included in Table 6.10 correspond to those adults aged 25 to 34 years, as it was the closest to the group studied in Limache.

Table 6.10: Mean daily macro and micronutrient intakes as percentages of recommended values in adults from Britain and Chile

		Chile *[% of EAR]		UK ^[% of EAR]	
		Males	**Females**	**Males**	**Females**
	Energy (Kcal)	2,324 [91.1]	1,668 [86.0]	2,313 [90.1]	1,640 [85.0]
Macronutrients	**% Contribution of macronutrients to energy intake in surveyed adults [% EAR]**				
	Proteins	14.5 [96.7]	13.9 [92.3]	16.5 [110.0]	16.6 [110.7]
	Carbohydrates	57 [114.0]	58 [116.0]	47.7 [95.4]	48.5 [97.0]
	Total Fat	28.0 [80.0]	28.0 [80.0]	35.8 [102.3]	34.9 [100.0]
	PUFA	N/A	N/A	N/A	N/A
	SFA	7.0 [63.4]	8.0 [72.7]	13.4 [121.8]	13.2 [120.0]
	MUFA	N/A	N/A	12.1 [93.1]	11.5 [104.5]
	Omega 3	N/A	N/A	1.0 [500.0]	1.0 [500.0]
	Omega 6	N/A	N/A	5.4 [540.0]	5.3 [540.0]
	Mean intakes as percentage of RNI in surveyed adults				
Vitamins	Vitamin A	87	102	80	78
	Vitamin C	165	160	162	170
	Vitamin E	400	300	N/A	N/A
	Folic acid	127	111	151	170
Minerals	Calcium	80	48.9	123	99
	Iron	150	100	131	60
	Phosphorus	N/A	N/A	243	190
	Magnesium	N/A	N/A	86	76
	Zinc	118.6[#]	194[#]	95	98
	Selenium	N/A	N/A	86	78

* Data from Chile obtained from ref. [307 ^ Data from Britain obtained from ref. [330]
# Data on zinc obtained from ref. [331]

British recommendations of protein are given in the form of EAR or RNI for men and women but not as percentage. However, the report of the British Survey informed protein intake only as -% contribution to energy intake. As their recommendation for fat is 35% TEI and carbohydrates 50% it was assumed that a 100% met recommended value for proteins would be a 15% of TEI.

In Chile, information on dietary intake in the general population is much more limited and it comes mainly from small studies, which may not always be representative and are usually designed to test hypotheses related to specific illnesses rather than just evaluate dietary intake on its own. The Chilean mean energy and nutrient intakes presented in Table 6.10 correspond to a survey carried out in Santiago, aimed to explore the dietary pattern of 859 adults aged 35.8± 6.9 years old attending any of 120 health care units of the Chilean public health system [307]. Data on zinc were not available in this study, and therefore were taken from an earlier study assessing zinc intake in a sample of 37 adults aged 20 to 35 years old from low socio-economic stratum living in Santiago [331]. These nutritional values are presented as percentage of the British RNI.

The two countries report a fairly similar dietary intake in terms of its nutritional adequacy, the Chilean population having a lower fraction intake of fats and higher in carbohydrates. The available comparable data on micronutrients suggests that both populations have an adequate intake of antioxidant vitamins and minerals, but that consumption of calcium is below the recommended values in Chilean females (Table 6.10). The participants of Limache had an intake that confirms these similarities and differences, although the intake of vitamin A was adequate and sufficient in the current study (Table 6.8).

Correlations between intake of macronutrients and those micronutrients included in the main analyses and TEI are presented in Table 6.11. All macro and micronutrients were statistically significantly correlated with TEI. Amongst macronutrients, the carbohydrates had the highest correlation with TEI. PUFA, MUFA and SFA also showed high correlations with TEI, but these were much weaker with omega 3 and omega 6. Vitamin E and the minerals selenium and zinc were highly correlated with

TEI. In contrast, vitamins A and C, carotene, retinol, and flavonoids were only weakly correlated with TEI.

Table 6.11: Correlation between macro and micronutrients and energy intake

Type of nutrient	Nutrient	Pearson's correlation with TEI [95% confidence interval]*
Macro-nutrients	Proteins	0.78 [0.76 to 0.80]
	Carbohydrates	0.94 [0.936 to 0.95]
	Lipids	0.87 [0.85 to 0.88]
	PUFA	0.75 [0.73 to 0.78]
	MUFA	0.83 [0.81 to 0.84]
	SFA	0.83 [0.81 to 0.84]
	Omega 6	0.41 [0.36 to 0.45]
	Omega 3	0.24 [0.19 to 0.30]
	Ratio omega 6/omega 3	-0.09 [-0.15 to –0.03]
Vitamins	Carotene	0.24 [0.18 to 0.29]
	Retinol	0.24[0.19 to 0.29]
	Total vitamin A	0.31 [0.26 to 0.36]
	Vitamin C	0.38 [0.33 to 0.42]
	Vitamin E	0.70 [0.67 to 0.73]
Minerals	Selenium	0.87 [0.85 to 0.88]
	Zinc	0.80 [0.78 to 0.82]
Flavonoids	Total catechins	0.27 [0.22 to 0.32]
	Flavonols	0.31 [0.26 to 0.36]
	Flavones	0.09 [0.03 to 0.14]

* All correlations showed a p value <0.001

Correlations between main antioxidant foods and nutrients and biomarkers of antioxidant status are summarised in Table 6.12. The measurement of FRAP captures the reductant capacity of vitamin C and E in the plasma, but in this study there were no correlations with either of these nutrients. A negative and statistically significant correlation was found between carrot and β–carotene (a major nutrient of carrot) with FRAP. In the case of uric acid, positive and statistically significant correlations were found with PUFA, omega 6, vitamin E, selenium, and zinc.

Table 6.12: Correlation between selected antioxidant food items and nutrients, and biomarkers of antioxidant status

Type of food/nutrient	Food item (g)	Pearson's correlation with biomarkers of antioxidant status [95% confidence interval]	
		FRAP	Uric Acid
Fruits	Orange	-0.06 [-0.14 to 0.02]	-0.01 [-0.09 to 0.07]
	Lemon	0.02 [-0.07 to 0.10]	0.05 [-0.03 to 0.13]
	Kiwi	-0.08 [-0.16 to 0.01]	-0.02 [-0.10 to 0.06]
	Apple	-0.001 [-0.08 to 0.08]	-0.05 [-0.13 to 0.04]
Vegetables	Garlic	-0.01 [-0.09 to 0.07]	0.02 [-0.06 to 0.10]
	Onion	-0.01 [-0.10 to 0.07]	0.04 [-0.04 to 0.12]
	Pumpkin	-0.07 [-0.15 to 0.01]	-0.05 [-0.13 to 0.03]
	Carrot	-0.11 [-0.19 to –0.03]*	-0.08 [-0.16 to 0.01]
	Avocado	-0.03 [-0.11 to 0.06]	0.07 [-0.02 to 0.15]
	Tea	-0.06 [-0.14 to 0.02]	-0.03 [-0.11 to 0.06]
Fatty acids	PUFA	-0.02 [-0.10 to 0.06]	0.11 [0.03 to 0.19]*
	MUFA	0.04 [-0.04 to 0.12]	0.07 [-0.01 to 0.15]
	SFA	0.07 [-0.02 to 0.15]	0.06 [-0.03 to 0.14]
	Omega 6	-0.04 [-0.13 to 0.04]	0.14 [0.06 to 0.22]^
	Omega 3	-0.01 [-0.09 to 0.08]	-0.01 [-0.09 to 0.07]
	Ratio omega 6/omega 3	-0.04 [-0.12 to 0.04]	0.02 [-0.06 to 0.10]
Antioxidant micro-nutrients	β–Carotene	-0.10 [-0.18 to –0.02]*	-0.06 [-0.14 to 0.03]
	Retinol	0.05 [-0.04 to 0.13]	-0.03 [-0.12 to 0.05]
	Total vitamin A	-0.06 [-0.14 to 0.03]	-0.07 [-0.15 to 0.02]
	Vitamin C	-0.06 [-0.14 to 0.02]	0.02 [-0.06 to 0.10]
	Vitamin E	-0.03 [-0.11 to 0.05]	0.12 [0.04 to 0.20]^
	Selenium	0.07 [-0.01 to 0.15]	0.12 [0.04 to 0.20]^
	Zinc	0.05 [-0.04 to 0.13]	0.13 [0.05 to 0.21]^

* $p = 0.01$ ^ $p \leq 0.0049$

CHAPTER 7
RESULTS II: Associations between respiratory symptoms, and BHR with dietary antioxidants, fatty acids and biomarkers

7.1 RESPIRATORY SYMPTOMS AND THEIR ASSOCIATION WITH FRUITS AND VEGETABLES, NUTRIENTS, FLAVONOIDS AND BIOMARKERS

7.1.1 Having wheeze in the last 12 months

Consumption of fruits and vegetables was unrelated to having wheeze in the last 12 months in the adults of Limache (Table 7.1). In relation to nutrient intake, the same lack of association was shown both univariately and after adjusting for potential confounders with most antioxidants, except for vitamin E, for which the ORs were consistently above 1.00 but the association was weakly statistically significant (Table 7.2). There was also no evidence for an association with any of the subclasses of flavonoids studied (Table 7.3). For biomarkers, a per quintile increase in the plasma levels of FRAP was negatively associated with the prevalence of wheeze in the last 12 months, being this association of borderline statistical significance (Table 7.4).

Table 7.1: Association between having wheeze in the last 12 months and food intake

Food group	Quintile groups	Unadjusted model		Adjusted model	
		Odds ratio (95% CI)	P-value (for trend)	Odds ratio (95% CI)	P-value (for trend)
Fruits (g)	1	1.00	0.13	1.00	0.24
	2	1.10 (0.75 to 1.63)		1.17 (0.78 to 1.75)	
	3	0.89 (0.60 to 1.32)		0.97 (0.64 to 1.47)	
	4	0.72 (0.48 to 1.09)		0.75 (0.49 to 1.15)	
	5	0.87 (0.58 to 1.30)		0.93 (0.61 to 1.42)	
Vegetables (g)	1	1.00	0.95	1.00	0.87
	2	0.98 (0.65 to 1.47)		1.00 (0.66 to 1.53)	
	3	1.28 (0.87 to 1.90)		1.33 (0.88 to 2.02)	
	4	0.90 (0.59 to 1.35)		0.94 (0.61 to 1.45)	
	5	1.03 (0.69 to 1.54)		1.08 (0.68 to 1.71)	

Table 7.2: Association between having wheeze in the last 12 months and nutrient intake

Nutrient	Quintile groups	Unadjusted model		Adjusted model	
		Odds ratio (95% CI)	P-value (for trend)	Odds ratio (95% CI)	P-value (for trend)
Vitamin C (mg)	1	1.00	0.28	1.00	0.35
	2	0.94 (0.63 to 1.40)		0.99 (0.66 to 1.50)	
	3	1.13 (0.77 to 1.68)		1.20 (0.80 to 1.80)	
	4	0.75 (0.50 to 1.14)		0.77 (0.50 to 1.18)	
	5	0.85 (0.57 to 1.27)		0.90 (0.57 to 1.40)	
Vitamin E (mg)	1	1.00	0.42	1.00	0.08
	2	1.09 (0.73 to 1.64)		1.22 (0.80 to 1.87)	
	3	1.07 (0.71 to 1.61)		1.22 (0.79 to 1.90)	
	4	1.11 (0.74 to 1.67)		1.30 (0.81 to 2.06)	
	5	1.24 (0.83 to 1.85)		1.68 (1.00 to 2.83)	
Total vitamin A (µg)	1	1.00	0.83	1.00	0.61
	2	0.79 (0.53 to 1.19)		0.89 (0.59 to 1.35)	
	3	0.85 (0.57 to 1.27)		0.94 (0.62 to 1.42)	
	4	0.96 (0.65 to 1.43)		1.00 (0.66 to 1.52)	
	5	0.97 (0.65 to 1.43)		1.07 (0.69 to 1.65)	
Omega 3 fatty acids (mg)	1	1.00	0.93	1.00	0.41
	2	1.18 (0.79 to 1.76)		1.21 (0.80 to 1.83)	
	3	1.12 (0.75 to 1.68)		1.23 (0.81 to 1.88)	
	4	1.16 (0.77 to 1.74)		1.30 (0.85 to 1.99)	
	5	1.06 (0.70 to 1.59)		1.18 (0.77 to 1.82)	
Ratio n6/n3	1	1.00	0.20	1.00	0.51
	2	1.43 (0.95 to 2.17)		1.46 (0.95 to 2.22)	
	3	1.42 (0.94 to 2.15)		1.42 (0.93 to 2.17)	
	4	1.55 (1.03 to 2.34)		1.51 (0.99 to 2.30)	
	5	1.26 (0.83 to 1.92)		1.16 (0.75 to 1.78)	
Selenium (µg)	1	1.00	0.42	1.00	0.89
	2	0.76 (0.51 to 1.14)		0.80 (0.53 to 1.23)	
	3	0.97 (0.65 to 1.43)		0.99 (0.64 to 1.54)	
	4	0.92 (0.62 to 1.37)		0.98 (0.59 to 1.63)	
	5	0.75 (0.62 to 1.37)		0.82 (0.42 to 1.58)	
Zinc (mg)	1	1.00	0.08	1.00	0.44
	2	0.77 (0.52 to 1.14)		0.83 (0.55 to 1.26)	
	3	0.77 (0.52 to 1.15)		0.88 (0.57 to 1.37)	
	4	0.69 (0.46 to 1.03)		0.74 (0.45 to 1.21)	
	5	0.76 (0.51 to 1.12)		0.85 (0.47 to 1.54)	

Table 7.3: Association between having wheeze in the last 12 months and flavonoid intake

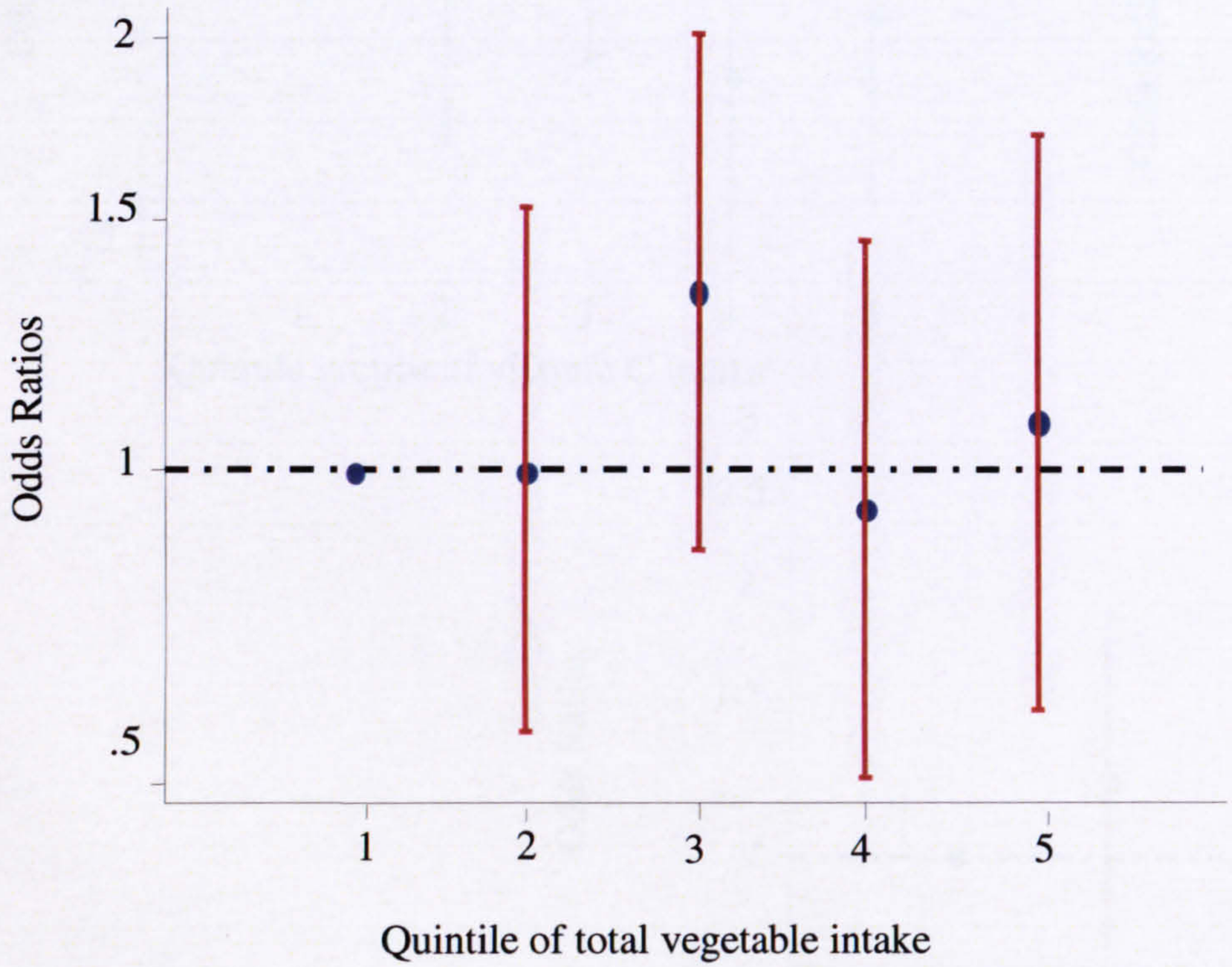
Flavonoids	Quintile groups	Unadjusted model		Adjusted model	
		Odds ratio (95% CI)	P-value (for trend)	Odds ratio (95% CI)	P-value (for trend)
Flavones (mg/d)	1	1.00	0.12	1.00	0.16
	2	0.96 (0.63 to 1.45)		0.95 (0.62 to 1.45)	
	3	1.19 (0.80 to 1.78)		1.21 (0.80 to 1.83)	
	4	1.02 (0.68 to 1.54)		0.98 (0.64 to 1.50)	
	5	1.37 (0.92 to 2.04)		1.37 (0.90 to 2.07)	
Flavonols (mg/d)	1	1.00	0.84	1.00	0.49
	2	0.85 (0.57 to 1.26)		0.79 (0.52 to 1.19)	
	3	0.93 (0.62 to 1.38)		0.88 (0.59 to 1.33)	
	4	0.83 (0.55 to 1.24)		0.75 (0.50 to 1.14)	
	5	0.97 (0.65 to 1.43)		0.87 (0.57 to 1.34)	
Total catechins (mg/d)	1	1.00	0.19	1.00	0.19
	2	0.94 (0.62 to 1.41)		1.06 (0.69 to 1.61)	
	3	0.86 (0.57 to 1.30)		0.95 (0.61 to 1.46)	
	4	0.98 (0.65 to 1.47)		0.99 (0.65 to 1.51)	
	5	1.31 (0.88 to 1.93)		1.40 (0.92 to 2.15)	

Table 7.4: Association between having wheeze in the last 12 months and plasma biomarkers

Plasma biomarker	Quintile groups	Unadjusted model		Adjusted model	
		Odds ratio (95% CI)	P-value (for trend)	Odds ratio (95% CI)	P-value (for trend)
FRAP (μM)	1	1.00	0.13	1.00	0.05
	2	1.09 (0.62 to 1.92)		1.08 (0.59 to 1.95)	
	3	1.23 (0.70 to 2.16)		1.17 (0.65 to 2.11)	
	4	0.80 (0.44 to 1.44)		0.73 (0.39 to 1.37)	
	5	0.69 (0.37 to 1.26)		0.57 (0.29 to 1.11)	
Uric Acid (mg/dL)	1	1.00	0.40	1.00	0.53
	2	1.22 (0.68 to 2.19)		1.13 (0.62 to 2.07)	
	3	0.93 (0.50 to 1.71)		0.93 (0.49 to 1.75)	
	4	1.23 (0.69 to 2.21)		1.21 (0.65 to 2.25)	
	5	1.32 (0.74 to 2.36)		1.21 (0.65 to 2.27)	
Protein Carbonyls (nm/mg protein)	1	1.00	0.24	1.00	0.23
	2	0.91 (0.51 to 1.62)		0.95 (0.52 to 1.72)	
	3	1.29 (0.74 to 2.27)		1.35 (0.75 to 2.41)	
	4	1.00 (0.56 to 1.78)		0.99 (0.55 to 1.78)	
	5	0.6 (0.33 to 1.14)		0.62 (0.33 to 1.17)	
F2-isoprostanes (pg/mL)	1	1.00	0.05	1.00	0.08
	2	0.74 (0.41 to 1.33)		0.65 (0.35 to 1.20)	
	3	0.19 (0.85 to 2.60)		1.52 (0.85 to 2.72)	
	4	0.83 (0.46 to 1.49)		0.83 (0.45 to 1.55)	
	5	0.43 (0.23 to 0.84)		0.43 (0.22 to 0.85)	

The associations between wheeze in the last 12 months (commonest reported symptom) with total fruit and vegetable intake, as well as with vitamins, the best described antioxidant nutrients in the literature, are illustrated in Figure 7.1 and 7.2, respectively.

FIGURE 7.1 ASSOCIATION BETWEEN WHEEZE IN THE LAST 12 MONTHS AND FOOD INTAKE



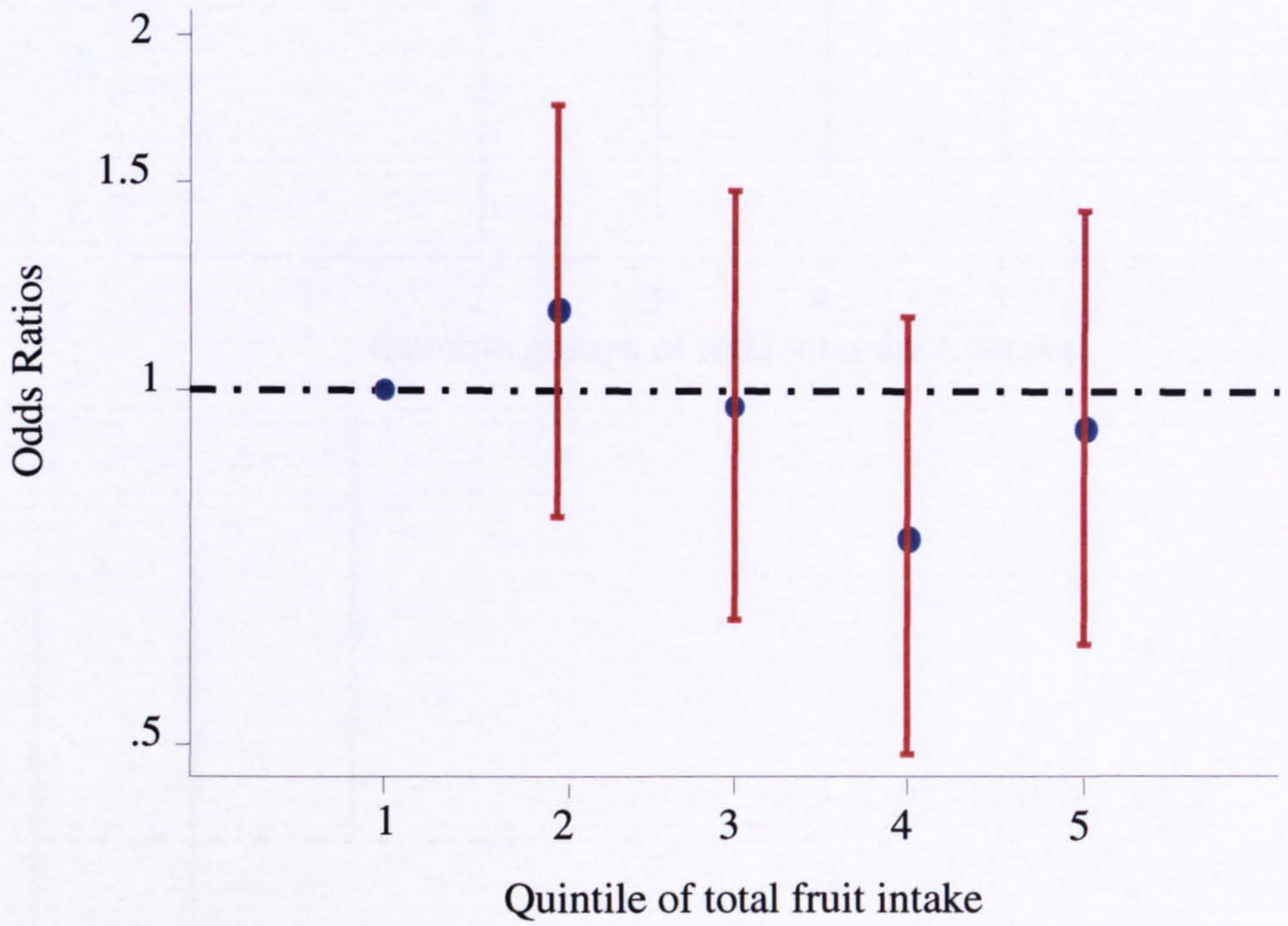
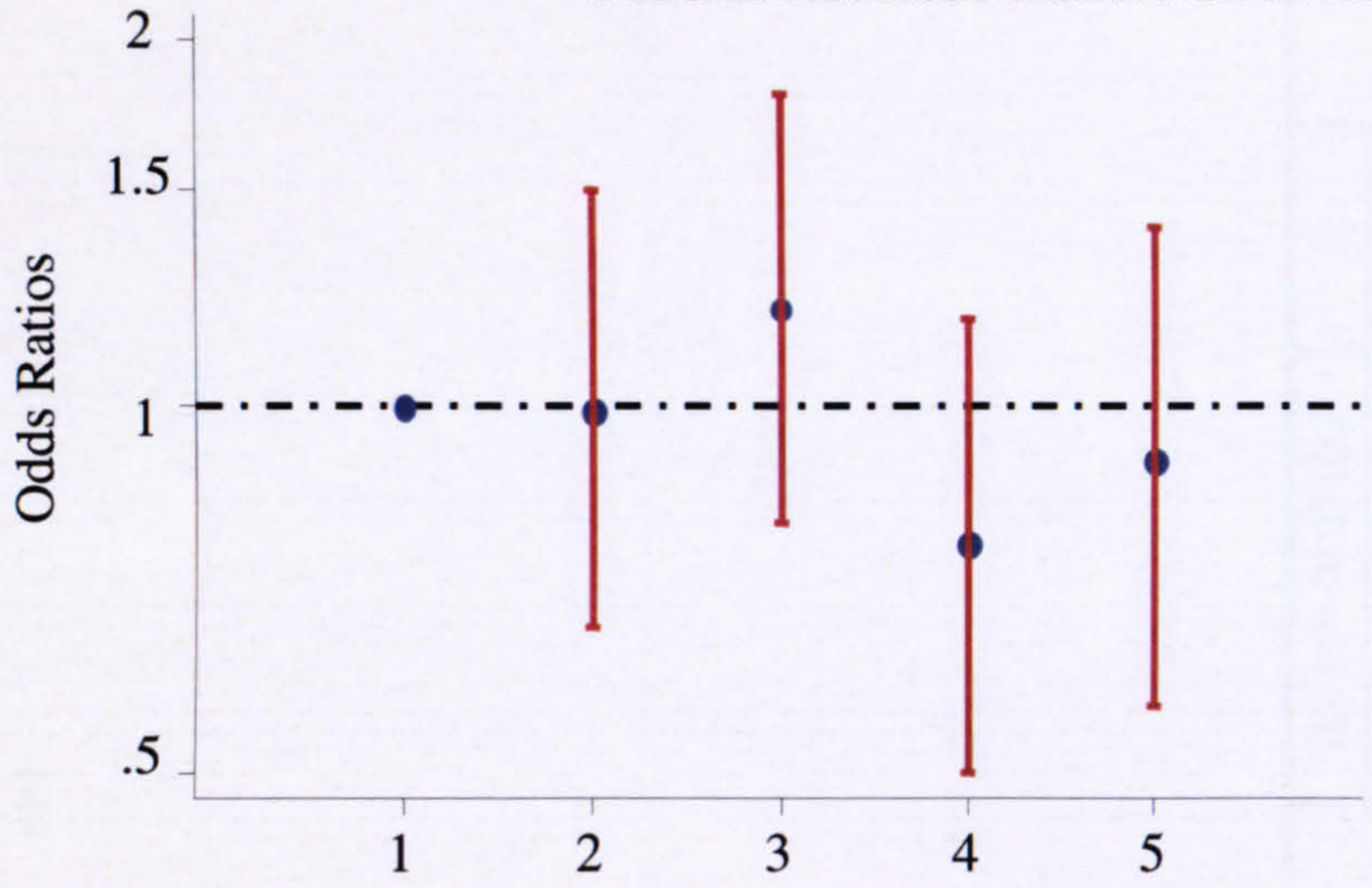
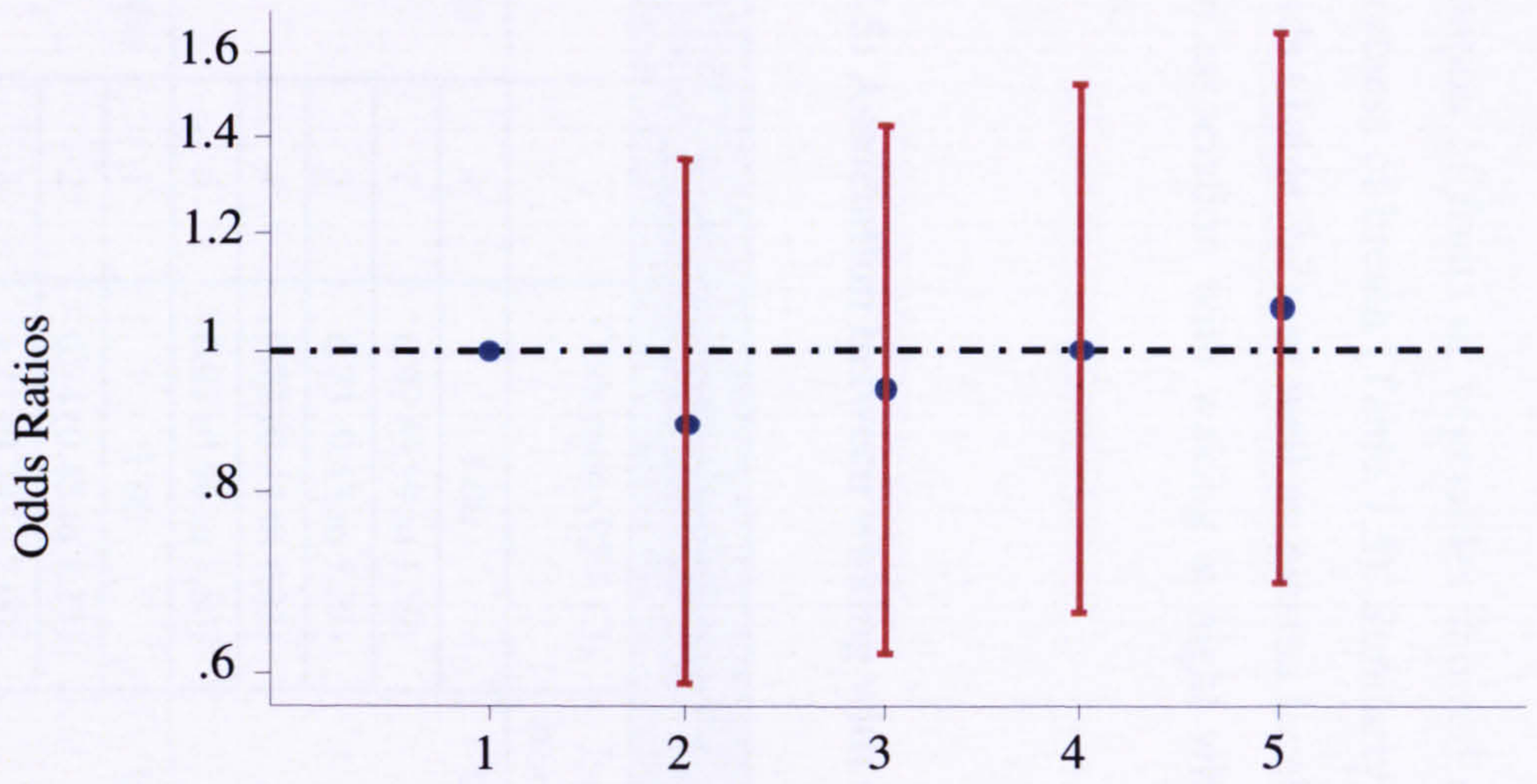
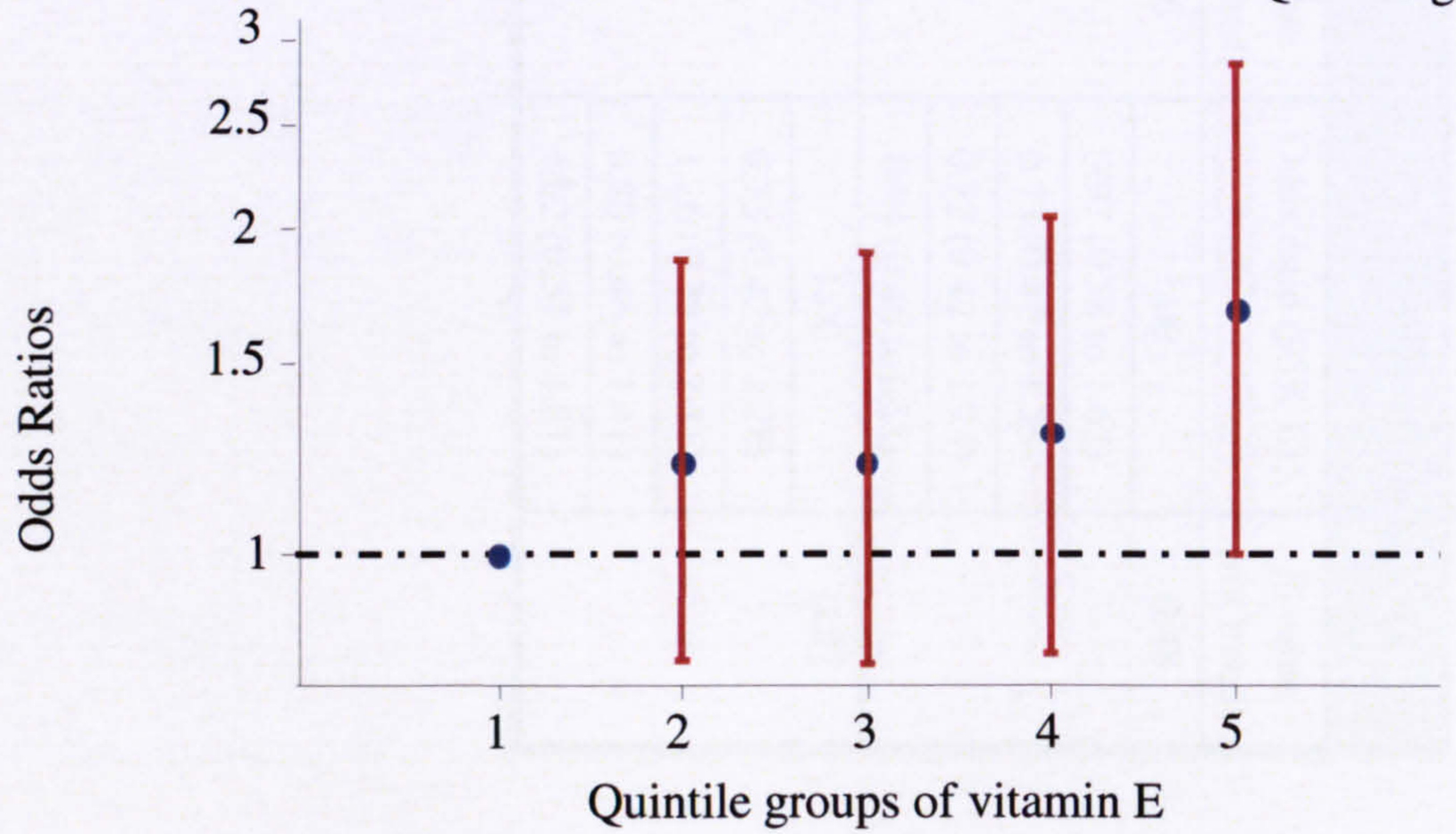


FIGURE 7.2: ASSOCIATION BETWEEN WHEEZE IN THE LAST 12 MONTHS AND ANTIOXIDANT VITAMINS







7.1.2 Waking at night with shortness of breath

Consumption of fruits or vegetables showed no association with waking at night with shortness of breath (Table 7.5). Similarly, intake of nutrients (Table 7.6), and flavonoids (Table 7.7), as well as plasma levels of biomarkers (Table 7.8), did not show an association with waking at night with shortness of breath in any of the analyses.

Table 7.5: Association between waking with shortness of breath and food intake

Food group	Quintile groups	Unadjusted model		Adjusted model	
		Odds ratio (95% CI)	P-value (for trend)	Odds ratio (95% CI)	P-value (for trend)
Fruits (g)	1	1.00	0.56	1.00	0.48
	2	0.97 (0.59 to 1.59)		0.97 (0.58 to 1.62)	
	3	0.73 (0.43 to 1.23)		0.73 (0.42 to 1.25)	
	4	0.72 (0.43 to 1.23)		0.72 (0.42 to 1.23)	
	5	0.97 (0.59 to 1.60)		0.94 (0.46 to1.58)	
Vegetables (g)	1	1.00	0.67	1.00	0.87
	2	0.74 (0.43 to 1.27)		0.73 (0.42 to 1.28)	
	3	1.21 (0.74 to 2.00)		1.26 (0.75 to 2.11)	
	4	0.83 (0.49 to 1.42)		0.80 (0.46 to 1.41)	
	5	1.07 (0.65 to 1.78)		1.02 (0.57 to 1.81)	

Table 7.6: Association between waking with shortness of breath and nutrient intake

Nutrient	Quintile groups	Unadjusted model		Adjusted model	
		Odds ratio (95% CI)	P-value (for trend)	Odds ratio (95% CI)	P-value (for trend)
Vitamin C (mg)	1	1.00	0.87	1.00	0.52
	2	0.97 (0.58 to 1.62)		0.94 (0.55 to 1.59)	
	3	0.97 (0.58 to 1.63)		0.92 (0.54 to 1.56)	
	4	0.90 (0.53 to 1.52)		0.79 (0.46 to 1.36)	
	5	1.00 (0.60 to 1.68)		0.89 (0.50 to 1.57)	
Vitamin E (mg)	1	1.00	0.82	1.00	0.96
	2	1.61 (0.96 to 2.69)		1.67 (0.98 to 2.83)	
	3	1.21 (0.71 to 2.08)		1.23 (0.70 to 2.17)	
	4	1.00 (0.57 to 1.75)		1.01 (0.54 to 1.87)	
	5	1.26 (0.73 to 2.15)		1.33 (0.67 to 2.61)	
Total vitamin A (µg)	1	1.00	0.94	1.00	0.65
	2	1.31 (0.79 to 2.18)		1.32 (0.79 to 2.23)	
	3	1.00 (0.59 to 1.71)		1.00 (0.58 to 1.72)	
	4	0.89 (0.52 to 1.54)		0.86 (0.49 to 1.51)	
	5	1.20 (0.71 to 2.01)		1.09 (0.62 to 1.91)	
Omega 3 fatty acids (mg)	1	1.00	0.55	1.00	0.42
	2	1.34 (0.78 to 2.29)		1.35 (0.78 to 2.33)	
	3	1.40 (0.82 to 2.39)		1.48 (0.85 to 2.56)	
	4	1.44 (0.84 to 2.45)		1.56 (0.90 to 2.72)	
	5	1.14 (0.65 to 1.98)		1.22 (0.68 to 2.18)	
Ratio n6/n3	1	1.00	0.41	1.00	0.34
	2	1.00 (0.60 to 1.69)		0.98 (0.58 to 1.66)	
	3	1.18 (0.71 to 1.96)		1.16 (0.70 to 1.93)	
	4	1.08 (0.64 to 1.80)		1.05 (0.63 to 1.77)	
	5	0.73 (0.42 to 1.27)		0.69 (0.39 to 1.22)	
Selenium (µg)	1	1.00	0.29	1.00	0.51
	2	1.19 (0.71 to 2.00)		1.11 (0.65 to 1.89)	
	3	1.48 (0.90 to 2.44)		1.38 (0.80 to 2.39)	
	4	1.04 (0.90 to 2.44)		0.93 (0.49 to 1.79)	
	5	0.72 (0.41 to 1.27)		0.58 (0.24 to 1.37)	
Zinc (mg)	1	1.00	0.38	1.00	0.64
	2	0.81 (0.49 to 1.36)		0.82 (0.48 to 1.40)	
	3	0.97 (0.59 to 1.60)		1.02 (0.59 to 1.75)	
	4	0.94 (0.57 to 1.55)		1.00 (0.55 to 1.82)	
	5	0.67 (0.39 to 1.15)		0.66 (0.31 to 1.44)	

Table 7.7: Association between waking with shortness of breath and flavonoid intake

Flavonoids	Quintile groups	Unadjusted model		Adjusted model	
		Odds ratio (95% CI)	P-value (for trend)	Odds ratio (95% CI)	P-value (for trend)
Flavones (mg/d)	1	1.00	0.80	1.00	0.88
	2	0.78 (0.46 to 1.32)		0.79 (0.46 to 1.34)	
	3	0.91 (0.54 to 1.51)		0.87 (0.52 to 1.47)	
	4	0.78 (0.46 to 1.32)		0.73 (0.43 to 1.25)	
	5	1.07 (0.65 to 1.76)		0.98 (0.43 to 1.25)	
Flavonols (mg/d)	1	1.00	0.28	1.00	0.37
	2	0.96 (0.57 to 1.64)		0.99 (0.44 to 1.36)	
	3	0.76 (0.43 to 1.33)		0.77 (0.78 to 2.21)	
	4	1.34 (0.81 to 2.21)		1.31 (0.78 to 2.21)	
	5	1.15 (0.69 to 1.93)		1.13 (0.65 to 1.96)	
Total catechins (mg/d)	1	1.00	0.28	1.00	0.18
	2	0.85 (0.49 to 1.48)		0.93 (0.77 to 2.31)	
	3	1.16 (0.69 to 1.95)		1.34 (0.77 to 2.31)	
	4	1.23 (0.73 to 2.06)		1.37 (0.80 to 2.35)	
	5	1.16 (0.69 to 1.95)		1.28 (0.74 to 2.23)	

Table 7.8: Association between waking with shortness of breath and plasma biomarkers

Plasma biomarker	Quintile groups	Unadjusted model		Adjusted model	
		Odds ratio (95% CI)	P-value (for trend)	Odds ratio (95% CI)	P-value (for trend)
FRAP (μM)	1	1.00	0.33	1.00	0.56
	2	0.93 (0.44 to 1.98)		0.92 (0.42 to 1.98)	
	3	0.86 (0.40 to 1.85)		0.93 (0.43 to 1.98)	
	4	1.00 (0.47 to 2.11)		1.06 (0.48 to 2.13)	
	5	0.59 (0.26 to 1.36)		0.66 (0.27 to 1.63)	
Uric Acid (mg/dL)	1	1.00	0.22	1.00	0.43
	2	0.66 (0.32 to 1.35)		0.67 (0.32 to 1.38)	
	3	0.39 (0.17 to 0.89)		0.41 (0.18 to 0.97)	
	4	0.38 (0.16 to 0.86)		0.41 (0.18 to 0.98)	
	5	0.78 (0.39 to 1.58)		0.88 (0.42 to 1.87)	
Protein Carbonyls (nm/mg protein)	1	1.00	0.65	1.00	0.65
	2	1.93 (0.85 to 4.37)		1.92 (0.84 to 4.42)	
	3	1.59 (0.68 to 3.70)		1.53 (0.65 to 3.60)	
	4	1.34 (0.56 to 3.18)		1.34 (0.56 to 3.23)	
	5	1.57 (0.68 to 3.66)		1.56 (0.66 to 3.66)	
F2-isoprostanes (pg/mL)	1	1.00	0.41	1.00	0.65
	2	1.65 (0.76 to 3.58)		1.74 (0.78 to 3.87)	
	3	1.29 (0.57 to 2.89)		1.42 (0.62 to 3.24)	
	4	1.13 (0.49 to 2.59)		1.17 (0.50 to 2.78)	
	5	0.83 (0.34 to 2.00)		0.97 (0.40 to 1.40)	

7.1.3 Having at least one respiratory symptom

There was no association between total intake of fruit or vegetables and having at least one respiratory symptom (Table 7.9). Analyses for intake of nutrient antioxidants and omega 3 fatty acid also showed no evidence for an association with this outcome (Table 7.10).

Amongst flavonoids, intake of total catechins showed a positive association with respiratory symptoms, which was of borderline nominal statistical significance in the univariate and adjusted model, but the Bonferroni-corrected P-value was above 0.1 (Table 7.11). No associations were observed for the other two groups of flavonoids studied.

The logistic regressions with plasma levels of biomarkers showed F-2isoprostanes had a nominally statistically significant negative association with having at least one respiratory symptom, but again the Bonferroni-corrected P-value was above 0.1 (Table 7.12).

Table 7.9: Association between having at least one respiratory symptom and food intake

Food group	Quintile groups	Unadjusted model		Adjusted model	
		Odds ratio (95% CI)	P-value (for trend)	Odds ratio (95% CI)	P-value (for trend)
Fruits (g)	1	1.00	0.17	1.00	0.14
	2	0.93 (0.65 to 1.34)		0.97 (0.67 to 1.41)	
	3	0.89 (0.62 to 1.28)		0.92 (0.63 to 1.34)	
	4	0.66 (0.45 to 0.95)		0.66 (0.45 to 0.96)	
	5	0.89 (0.62 to 1.28)		0.89 (0.60 to 1.30)	
Vegetables (g)	1	1.00	0.78	1.00	0.96
	2	0.85 (0.59 to 1.23)		0.84 (0.58 to 1.23)	
	3	1.14 (0.79 to 1.63)		1.14 (0.78 to 1.66)	
	4	0.89 (0.61 to 1.28)		0.89 (0.59 to 1.29)	
	5	1.04 (0.72 to 1.50)		1.00 (0.65 to 1.51)	

Table 7.10: Association between having at least one respiratory symptom and nutrient intake

Nutrient	Quintile groups	Unadjusted model		Adjusted model	
		Odds ratio (95% CI)	P-value (for trend)	Odds ratio (95% CI)	P-value (for trend)
Vitamin C (mg)	1	1.00	0.47	1.00	0.13
	2	0.76 (0.53 to 1.09)		0.75 (0.52 to 1.10)	
	3	0.99 (0.69 to 1.42)		0.96 (0.66 to 1.40)	
	4	0.69 (0.48 to 1.00)		0.63 (0.43 to 0.93)	
	5	0.86 (0.60 to 1.24)		0.78 (0.52 to 1.17)	
Vitamin E (mg)	1	1.00	0.53	1.00	0.29
	2	1.04 (0.72 to 1.49)		1.11 (0.76 to 1.62)	
	3	0.99 (0.69 to 1.43)		1.05 (0.71 to 1.56)	
	4	1.11 (0.77 to 1.60)		1.18 (0.78 to 1.78)	
	5	1.12 (0.78 to 1.61)		1.31 (0.82 to 2.11)	
Total vitamin A (µg)	1	1.00	0.99	1.00	0.73
	2	0.79 (0.55 to 1.13)		0.83 (0.57 to 1.22)	
	3	0.88 (0.61 to 1.26)		0.90 (0.62 to 1.32)	
	4	0.79 (0.55 to 1.13)		0.76 (0.52 to 1.12)	
	5	0.99 (0.69 to 1.42)		0.97 (0.65 to 1.44)	
Omega 3 fatty acids (mg)	1	1.00	0.45	1.00	0.21
	2	1.07 (0.74 to 1.54)		1.09 (0.75 to 1.58)	
	3	1.35 (0.94 to 1.94)		1.48 (1.02 to 2.16)	
	4	1.13 (0.78 to 1.63)		1.27 (0.86 to 1.86)	
	5	1.11 (0.77 to 1.60)		1.23 (0.83 to 1.81)	
Ratio n6/n3	1	1.00	0.71	1.00	0.98
	2	1.16 (0.80 to 1.67)		1.14 (0.78 to 1.66)	
	3	1.32 (0.92 to 1.90)		1.31 (0.90 to 1.90)	
	4	1.38 (0.95 to 1.98)		1.33 (0.92 to 1.94)	
	5	1.01 (0.70 to 1.46)		0.92 (0.63 to 1.35)	
Selenium (µg)	1	1.00	0.44	1.00	0.997
	2	0.95 (0.66 to 1.37)		0.98 (0.67 to 1.43)	
	3	1.07 (0.74 to 1.52)		1.11 (0.74 to 1.66)	
	4	1.03 (0.72 to 1.45)		1.11 (0.70 to 1.76)	
	5	0.79 (0.54 to 1.14)		0.84 (0.46 to 1.52)	
Zinc (mg)	1	1.00	0.18	1.00	0.53
	2	0.80 (0.56 to 1.15)		0.86 (0.59 to 1.25)	
	3	0.79 (0.55 to 1.14)		0.87 (0.58 to 1.30)	
	4	0.93 (0.65 to 1.34)		0.99 (0.64 to 1.54)	
	5	0.70 (0.49 to 1.01)		0.72 (0.42 to 1.25)	

Table 7.11: Association between having at least one respiratory symptom and flavonoid intake

Flavonoids	Quintile groups	Unadjusted model		Adjusted model		
		Odds ratio (95% CI)	P-value (for trend)	Odds ratio (95% CI)	P-value (for trend)	P-value (Bonferroni corrected)
Flavones (mg/d)	1	1.00	0.14	1.00	0.31	
	2	0.81 (0.56 to 1.17)		0.81 (0.55 to 1.17)		
	3	1.02 (0.71 to1.47)		1.01 (0.70 to 1.47)		
	4	0.92 (0.64 to 1.32)		0.86 (0.59 to 1.26)		
	5	1.28 (0.89 to 1.84)		1.20 (0.82 to 1.76)		
Flavonols (mg/d)	1	1.00	0.59	1.00	0.98	
	2	0.83 (0.57 to 1.19)		0.79 (0.54 to 1.15)		
	3	0.89 (0.62 to 1.28)		0.86 (0.59 to 1.26)		
	4	1.02 (0.71 to 1.46)		0.94 (0.65 to 1.38)		
	5	1.01 (0.70 to 1.45)		0.92 (0.62 to 1.36)		
Total catechins (mg/d)	1	1.00	0.05	1.00	0.04	0.12
	2	1.05 (0.73 to 1.52)		1.17 (0.80 to 1.71)		
	3	0.92 (0.64 to 1.33)		1.01 (0.68 to 1.49)		
	4	1.21 (0.84 to 1.74)		1.26 (0.86 to 1.85)		
	5	1.40 (0.97 to 2.01)		1.51(1.02 to 2.24)		

Table 7.12: Association between having at least one respiratory symptom and plasma levels of biomarkers

Plasma biomarker	Quintile groups	Unadjusted model		Adjusted model		
		Odds ratio (95% CI)	P-value (for trend)	Odds ratio (95% CI)	P-value (for trend)	P-value (Bonferroni corrected)
FRAP (μM)	1	1.00	0.06	1.00	0.08	
	2	1.19 (0.71 to 2.01)		1.15 (0.67 to 1.98)		
	3	1.37 (0.81 to 2.30)		1.40 (0.82 to 2.39)		
	4	0.83 (0.49 to 1.42)		0.83 (0.47 to 1.45)		
	5	0.66 (0.38 to 1.13)		0.64 (0.35 to 1.15)		
Uric Acid (mg/dL)	1	1.00	0.14	1.00	0.19	
	2	0.64 (0.38 to 1.08)		0.60 (0.35 to 1.02)		
	3	0.45 (0.26 to 0.76)		0.44 (0.25 to 0.76)		
	4	0.56 (0.33 to 0.95)		0.56 (0.32 to 0.98)		
	5	0.69 (0.41 to 1.16)		0.68 (0.39 to 1.18)		
Protein Carbonyls (nm/mg protein)	1	1.00	0.61	1.00	0.60	
	2	0.86 (0.51 to 1.44)		0.89 (0.52 to 1.52)		
	3	0.85 (0.50 to 1.43)		0.84 (0.49 to 1.44)		
	4	0.90 (0.53 to 1.52)		0.87 (0.51 to 1.49)		
	5	0.84 (0.50 to 1.41)		0.86 (0.50 to 1.47)		
F2-isoprostanes (pg/mL)	1	1.00	0.008	1.00	0.03	0.12
	2	0.92 (0.55 to 1.56)		0.84 (0.49 to 1.46)		
	3	1.11 (0.66 to 1.88)		1.17 (0.68 to 2.01)		
	4	0.78 (0.45 to 1.33)		0.79 (0.45 to 1.39)		
	5	0.46 (0.26 to 0.80)		0.49 (0.28 to 0.88)		

7.2 BHR SLOPE AND ITS ASSOCIATION WITH FRUITS AND VEGETABLES, NUTRIENTS, FLAVONOIDS AND BIOMARKERS.

Analyses with BHR slope were carried out in 1,106 subjects. Univariate analyses showed a non-statistically significant trend for a negative association between both total fruit and vegetable intakes with BHR slope. Adjusted multiple linear regression did not change the direction or statistical significance of the relations (Table 7.13).

Table 7.13: Association between BHR (mg^{-1}) and food intake

Food group	Quintile groups	Unadjusted model		Adjusted model	
		Difference of means (95% CI)	P-value (for trend)	Difference of means (95% CI)	P-value (for trend)
Fruits (g)	1	0	0.42	0	0.17
	2	-0.19 (-0.48 to 0.10)		-0.19 (-0.49 to 0.10)	
	3	-0.25 (-0.54 to 0.04)		-0.27 (-0.51 to 0.08)	
	4	-0.17 (-0.46 to 0.12)		-0.21 (-0.53 to 0.08)	
	5	-0.18 (-0.47 to 0.11)		-0.22 (-0.53 to 0.08)	
Vegetables (g)	1	0	0.41	0	0.20
	2	-0.18 (-0.47 to 0.11)		-0.24 (-0.54 to 0.05)	
	3	-0.04 (-0.33 to 0.25)		-0.09 (-0.38 to 0.21)	
	4	-0.15 (-0.43 to 0.14)		-0.21 (-0.52 to 0.09)	
	5	-0.15 (-0.44 to 0.14)		-0.25 (-0.58 to 0.07)	

Per-quintile increase in vitamin C intake was negatively related to BHR in the adjusted model (Table 7.14) and selenium was positively associated with BHR. After Bonferroni correction, the association with vitamin C was no longer statistically significant, while there was still evidence for an increase in BHR with selenium intake.

Intake of flavonoids was unrelated to BHR slope in this population. Multivariable analyses showed negative associations with flavonols and total catechins, but they were not statistically significant (Table 7.15). In relation to plasma biomarkers, a similar negative association was observed with F2-ip but did not reach statistical significance (Table 7.16).

Table 7.14: Association between BHR (mg^{-1}) and nutrient intake

Nutrient	Quintile groups	Unadjusted model		Adjusted model		
		Difference of means (95% CI)	P-value (for trend)	Difference of means (95% CI)	P-value (for trend)	P-value (Bonferroni corrected)
Vitamin C (mg)	1	0	0.16	0	0.04	0.28
	2	-0.16 (-0.45 to 0.12)		-0.18 (-0.37 to 0.05)		
	3	-0.22 (-0.51 to 0.07)		-0.27 (-0.57 to 0.02)		
	4	-0.17 (-0.46 to 0.11)		-0.23 (-0.52 to 0.07)		
	5	-0.22 (-0.51 to 0.06)		-0.35 (-0.67 to -0.04)		
Vitamin E (mg)	1	0	0.88	0	0.56	
	2	0.08 (-0.21 to 0.37)		0.06 (-0.23 to 0.36)		
	3	0.12 (-0.17 to 0.41)		0.08 (-0.22 to 0.38)		
	4	0.10 (-0.19 to 0.39)		0.04 (-0.28 to 0.37)		
	5	-0.04 (-0.33 to 0.25)		-0.14 (-0.51 to 0.23)		
Total vitamin A (µg)	1	0	0.61	0	0.68	
	2	-0.06 (-0.35 to 0.22)		-0.04 (-0.33 to 0.26)		
	3	0.16 (-0.12 to 0.45)		0.17 (-0.12 to 0.47)		
	4	-0.07 (-0.36 to 0.22)		-0.10 (-0.40 to 0.20)		
	5	0.09 (-0.20 to 0.38)		0.10 (-0.21 to 0.41)		
Omega 3 fatty acids (mg)	1	0	0.43	0	0.42	
	2	-0.09 (-0.38 to 0.20)		-0.08 (-0.37 to 0.21)		
	3	0.05 (-0.24 to 0.34)		0.03 (-0.26 to 0.33)		
	4	0.08 (-0.21 to 0.37)		0.08 (-0.22 to 0.38)		
	5	0.05 (-0.24 to 0.34)		0.06 (-0.24 to 0.36)		
Ratio n6/n3	1	0	0.28	0	0.33	
	2	0.21 (-0.08 to 0.49)		0.20 (-0.09 to 0.49)		
	3	0.21 (-0.08 to 0.50)		0.17 (-0.12 to 0.46)		
	4	-0.08 (-0.37 to 0.21)		-0.07 (-0.36 to 0.22)		
	5	-0.03 (-0.32 to 0.26)		-0.03 (-0.32 to 0.26)		
Selenium (µg)	1	0	0.05	0	0.002	0.01
	2	0.32 (0.03 to 0.61)		0.38 (0.08 to 0.67)		
	3	0.23 (-0.06 to 0.51)		0.41 (0.09 to 0.72)		
	4	0.13 (-0.16 to 0.42)		0.41 (0.05 to 0.77)		
	5	0.41 (0.13 to 0.70)		0.89 (0.43 to 1.34)		
Zinc (mg)	1	0	0.47	0	0.40	
	2	0.01 (-0.28 to 0.30)		0.05 (-0.25 to 0.34)		
	3	0.18 (-0.11 to 0.47)		0.21 (-0.11 to 0.53)		
	4	0.13 (-0.16 to 0.42)		0.16 (-0.19 to 0.50)		
	5	0.06 (-0.23 to 0.35)		0.13 (-0.30 to 0.55)		

Table 7.15: Association between BHR (mg^{-1}) and flavonoid intake

Flavonoids	Quintile groups	Unadjusted model		Adjusted model	
		Difference of means (95% CI)	P-value (for trend)	Difference of means (95% CI)	P-value (for trend)
Flavones (mg/d)	1	0	0.29	0	0.39
	2	-0.02 (-0.31 to 0.27)		-0.02 (-0.31 to 0.27)	
	3	0.08 (-0.21 to 0.37)		0.08 (-0.21 to 0.37)	
	4	0.01 (-0.28 to 0.30)		-0.01 (-0.31 to 0.28)	
	5	0.16 (-0.13 to 0.45)		0.14 (-0.16 to 0.44)	
Flavonols (mg/d)	1	0	0.97	0	0.94
	2	-0.11 (-0.40 to 0.18)		-0.10 (-0.39 to 0.19)	
	3	-0.20 (-0.49 to 0.09)		-0.18 (-0.47 to 0.12)	
	4	-0.01 (-0.30 to 0.28)		-0.002 (-0.30 to 0.30)	
	5	-0.04 (-0.33 to 0.25)		-0.04 (-0.35 to 0.27)	
Total catechins (mg/d)	1	0	0.57	0	0.44
	2	-0.12 (-0.41 to 0.17)		-0.15 (-0.44 to 0.15)	
	3	0.02 (-0.27 to 0.31)		-0.01 (-0.31 to 0.29)	
	4	-0.06 (-0.35 to 0.23)		-0.10 (-0.40 to 0.20)	
	5	-0.13 (-0.16 to 0.25)		-0.16 (-0.46 to 0.15)	

Table 7.16: Association between BHR (mg^{-1}) and plasma levels of biomarkers

Plasma biomarker	Quintile group	Unadjusted model		Adjusted model	
		Difference of means (95% CI)	P-value (for trend)	Difference of means (95% CI)	P-value (for trend)
FRAP (μM)	1	0	0.70	0	0.79
	2	0.21 (-0.23 to 0.65)		0.17 (-0.28 to 0.62)	
	3	-0.12 (-0.56 to 0.32)		-0.15 (-0.60 to 0.30)	
	4	0.01 (-0.43 to 0.46)		0.03 (-0.43 to 0.49)	
	5	-0.001 (-0.44 to 0.44)		0.001 (-0.48 to 0.48)	
Uric Acid (mg/dL)	1	0	0.89	0	0.65
	2	0.12 (-0.32 to 0.56)		0.15 (-0.29 to 0.59)	
	3	0.15 (-0.29 to 0.60)		0.20 (-0.26 to 0.65)	
	4	-0.05 (-0.49 to 0.40)		-0.01 (-0.47 to 0.45)	
	5	0.12 (-0.33 to 0.56)		0.20 (0.27 to 0.67)	
Protein Carbonyls (nm/mg protein)	1	0	0.82	0	0.64
	2	0.23 (-0.21 to 0.67)		0.29 (-0.15 to 0.74)	
	3	0.23 (-0.21 to 0.67)		0.21 (-0.24 to 0.66)	
	4	0.19 (-0.25 to 0.63)		0.17 (-0.27 to 0.61)	
	5	-0.05 (-0.49 to 0.40)		-0.06 (-0.51 to 0.38)	
F2-isoprostanes (pg/mL)	1	0	0.30	0	0.41
	2	-0.02 (-0.47 to 0.43)		-0.15 (-0.62 to 0.31)	
	3	-0.25 (-0.70 to 0.21)		-0.31 (-0.78 to 0.15)	
	4	-0.51 (-0.97 to -0.04)		-0.57 (-1.04 to -1.00)	
	5	-0.03 (-0.49 to 0.43)		-0.02 (-0.48 to 0.45)	

CHAPTER 8
RESULTS III: Associations between lung function with dietary antioxidants, fatty acids and biomarkers

8.1 FEV_1 AND FEV_1/FVC AND THEIR ASSOCIATION WITH FRUITS AND VEGETABLES, NUTRIENTS AND PLASMA BIOMARKERS

Table 8.1 shows the association between FEV_1 and fruit and vegetable intake. The unadjusted analysis showed that total intake of vegetables was positively related to a greater FEV_1, which disappeared after adjustment for potential confounders. Analyses with individual food items (Appendix 3) showed that intake of garlic and onion was positively associated with a greater FEV_1 but this association lost statistical significance after Bonferroni-correction. In relation to nutrient intake (Table 8.2), univariate analyses showed a statistically significant association between FEV_1 and intake of vitamin E, omega 3 fatty acids, selenium, and zinc. After controlling for confounders, only intake of omega 3 fatty acids remained statistically significantly associated with a greater FEV_1. However, Bonferroni correction raised the P-value above 0.1.

Table 8.1: Association between best FEV_1 (L) and food intake

Food group	Quintile groups	Unadjusted model		Adjusted model	
		Difference of means (95% CI)	P-value (for trend)	Difference of means (95% CI)	P-value (for trend)
Fruits (g)	1	0	0.07	0	0.09
	2	0.05 (-0.07 to 0.17)		-0.02 (-0.09 to 0.05)	
	3	0.04 (-0.09 to 0.16)		0.03 (-0.04 to 0.10)	
	4	0.02 (-0.10 to 0.14)		0.02 (-0.05 to 0.09)	
	5	0.14 (0.02 to 0.27)		0.05 (-0.02 to 0.12)	
Vegetables (g)	1	0	<0.001	0	0.74
	2	0.16 (0.04 to 0.28)		0.03 (-0.04 to 0.10)	
	3	0.25 (0.12 to 0.37)		0.01 (-0.06 to 0.08)	
	4	0.26 (0.13 to 0.38)		0.01 (-0.06 to 0.08)	
	5	0.31 (0.18 to 0.43)		0.03 (-0.05 to 0.10)	

Table 8.2: Association between best FEV_1 (L) and nutrient intake

Nutrient	Quintile groups	Unadjusted model		Adjusted model		
		Difference of means (95% CI)	P-value (for trend)	Difference of means (95% CI)	P-value (for trend)	P-value (Bonferroni corrected)
Vitamin C (mg)	1	0	0.17	0	0.75	
	2	0.05 (-0.07 to 0.17)		-0.05 (-0.12 to 0.01)		
	3	0.07 (-0.05 to 0.19)		-0.002 (-0.07 to 0.07)		
	4	0.05 (-0.07 to 0.18)		0.004 (-0.06 to 0.07)		
	5	0.09 (-0.03 to 0.22)		-0.02 (-0.09 to 0.06)		
Vitamin E (mg)	1	0	<0.001	0	0.61	
	2	0.05 (-0.07 to 0.17)		-0.06 (-0.13 to 0.01)		
	3	0.16 (0.04 to 0.28)		-0.004 (-0.08 to 0.07)		
	4	0.29 (0.17 to 0.41)		-0.02 (-0.09 to 0.06)		
	5	0.46 (0.34 to 0.58)		0.01 (-0.08 to 0.09)		
Total vitamin A (µg)	1	0	0.94	0	0.78	
	2	0.09 (-0.03 to 0.21)		0.06 (-0.004 to 0.13)		
	3	0.07 (-0.05 to 0.19)		0.04 (-0.03 to 0.11)		
	4	0.05 (-0.07 to 0.18)		0.01 (-0.06 to 0.08)		
	5	0.02 (-0.10 to 0.15)		0.02 (-0.06 to 0.09)		
Omega 3 fatty acids (mg)	1	0	<0.001	0	0.02	0.14
	2	0.05 (-0.07 to 0.17)		0.04 (-0.03 to 0.11)		
	3	0.12 (0.001 to 0.25)		0.05 (-0.02 to 0.11)		
	4	0.24 (0.12 to 0.36)		0.08 (0.01 to 0.14)		
	5	0.25 (0.13 to 0.37)		0.08 (0.005 to 0.15)		
Ratio n6/n3	1	0	0.26	0	0.11	
	2	-0.02 (-0.14 to 0.11)		-0.01 (-0.07 to 0.06)		
	3	0.004 (-0.12 to 0.13)		-0.03 (-0.10 to 0.04)		
	4	-0.04 (-0.16 to 0.08)		0.01 (-0.06 to 0.08)		
	5	-0.07 (-0.19 to 0.06)		-0.07 (-0.14 to -0.005)		
Selenium (µg)	1	0	<0.001	0	0.30	
	2	0.08 (0.02 to 0.25)		-0.01 (-0.08 to 0.06)		
	3	0.26 (0.15 to 0.37)		-0.04 (-0.11 to 0.03)		
	4	0.48 (0.37 to 0.60)		-0.06 (-0.14 to 0.03)		
	5	0.75 (0.63 to 0.86)		-0.02 (-0.13 to 0.09)		
Zinc (mg)	1	0	0.02	0	0.82	
	2	0.14 (0.02 to 0.25)		0.02 (-0.05 to 0.09)		
	3	0.30 (0.19 to 0.42)		-0.05 (-0.12 to 0.03)		
	4	0.54 (0.42 to 0.65)		-0.01 (-0.09 to 0.07)		
	5	0.68 (0.57 to 0.80)		0.01 (-0.09 to 0.11)		

Intake of the three major classes of flavonoids studied was statistically significantly associated with a greater FEV_1 in the unadjusted analyses. After controlling for potential confounders, the association with flavones disappeared but it remained of borderline statistical significance for flavonols and total catechins (Table 8.3). Among plasma levels of biomarkers, a quintile increase in the levels of FRAP was related to a reduction of FEV_1, association that remained statistically significant after Bonferroni correction (Table 8.4).

Table 8.3: Association between best FEV_1 (L) and flavonoid intake

Flavonoids	Quintile groups	Unadjusted model		Adjusted model	
		Difference of means (95% CI)	P-value (for trend)	Difference of means (95% CI)	P-value (for trend)
Flavones (mg/d)	1	0	0.001	0	0.96
	2	-0.001 (-0.12 to 0.13)		0.01 (-0.05 to 0.08)	
	3	-0.12 (-0.24 to 0.002)		0.02 (-0.05 to 0.09)	
	4	-0.12 (-0.25 to -0.002)		-0.002 (-0.07 to 0.07)	
	5	-0.17 (-0.27 to -0.002)		0.01 (-0.06 to 0.07)	
Flavonols (mg/d)	1	0	<0.001	0	0.08
	2	0.10 (-0.02 to 0.23)		0.07 (0.003 to 0.14)	
	3	0.11 (-0.01 to 0.23)		0.05 (-0.02 to 0.12)	
	4	0.10 (-0.03 to 0.22)		0.05 (-0.02 to 0.12)	
	5	0.25 (0.13 to 0.37)		0.08 (0.01 to 0.16)	
Total catechins (mg/d)	1	0	<0.001	0	0.06
	2	0.14 (0.02 to 0.26)		-0.04 (-0.11 to 0.03)	
	3	0.35 (0.23 to 0.47)		0.03 (-0.03 to 0.11)	
	4	0.33 (0.20 to 0.45)		0.04 (-0.03 to 0.11)	
	5	0.35 (0.23 to 0.47)		0.04 (-0.03 to 0.11)	

In relation to the ratio FEV_1/FVC, there was no evidence of association with fruit intake. Consumption of total vegetables was negatively associated with FEV_1/FVC in the univariate model as the quintile consumption increased. Such association became weaker after adjusting for all potential confounders (Table 8.5).

Consumption of vitamin E, selenium and zinc was negatively associated with this outcome as shown by the difference of means in the crude model. After controlling for confounders, the association found with vitamin E intake was no longer observed, whereas that with selenium and zinc still showed a negative trend but the associations were not statistically significant (Table 8.6). The analyses with flavonoids showed that only total intake of catechins were associated with FEV_1/FVC. In the univariate model this association was highly statistically significant, but after controlling for confounders remained negative but it became much weaker (Table 8.7). Plasma biomarkers were unrelated to FEV_1/FVC (Table 8.8).

Table 8.4: Association between best FEV_1 (L) and plasma biomarkers

Plasma biomarker	Quintile groups	Unadjusted model		Adjusted model		
		Difference of means (95% CI)	P-value (for trend)	Difference of means (95% CI)	P-value (for trend)	P-value (Bonferroni corrected)
FRAP (μM)	1	0	<0.001	0	0.01	0.04
	2	0.20 (0.02 to 0.37)		0.06 (-0.04 to 0.15)		
	3	0.28 (0.11 to 0.45)		0.02 (-0.08 to 0.11)		
	4	0.44 (0.26 to 0.61)		-0.01 (-0.09 to 0.11)		
	5	0.40 (0.23 to 0.57)		-0.13 (-0.23 to -0.03)		
Uric Acid (mg/dL)	1	0	<0.001	0	0.66	
	2	0.08 (-0.09 to 0.25)		0.01 (-0.08 to 0.11)		
	3	0.28 (0.11 to 0.45)		0.08 (-0.02 to 0.18)		
	4	0.41 (0.24 to 0.58)		0.03 (-0.06 to 0.13)		
	5	0.38 (0.21 to 0.55)		-0.04 (-0.14 to 0.07)		
Protein Carbonyls (nm/mg protein)	1	0	0.09	0	0.94	
	2	0.05 (-0.12 to 0.23)		0.03 (-0.06 to 0.13)		
	3	0.06 (-0.12 to 0.24)		0.01 (-0.09 to 0.11)		
	4	0.11 (-0.06 to 0.29)		-0.01 (-0.11 to 0.09)		
	5	0.14 (-0.04 to 0.31)		0.02 (-0.08 to 0.12)		
F2-isoprostanes (pg/mL)	1	0	0.01	0	0.67	
	2	0.18 (0.01 to 0.36)		0.09 (-0.005 to 0.19)		
	3	0.28 (0.10 to 0.46)		0.07 (-0.03 to 0.16)		
	4	0.11 (-0.07 to 0.29)		-0.01 (-0.11 to 0.09)		
	5	0.29 (0.11 to 0.46)		0.07 (-0.02 to 0.17)		

Table 8.5: Association between ratio FEV_1/FVC and food intake

Food group	Quintile groups	Unadjusted model		Adjusted model	
		Difference in means (95% CI)	P-value (for trend)	Difference in means (95% CI)	P-value (for trend)
Fruits (g)	1	0	0.25	0	0.23
	2	-0.002 (-0.01 to 0.01)		-0.004 (-0.01 to 0.01)	
	3	0.001 (-0l01 to 0.01)		-0.002 (-0.01 to 0.004)	
	4	-0.003 (-0.01 to 0.01)		-0.005 (-0.01 to 0.004)	
	5	-0.01 (-0.01 to 0.003)		-0.005 (-0.01 to 0.003)	
Vegetables (g)	1	0	0.003	0	0.10
	2	0.002 (-0.01 to 0.01)		0.004 (-0.01 to 0.01)	
	3	-0.01 (-0.02 to -0.001)		-0.01 (-0.02 to 0.002)	
	4	-0.01 (-0.02 to -0.003)		-0.01 (-0.02 to 0.0002)	
	5	-0.01 (-0.02 to 0.001)		-0.002 (-0.01 to 0.007)	

Table 8.6: Association between ratio FEV_1/FVC and nutrient intake

Nutrient	Quintile groups	Unadjusted model		Adjusted model	
		Difference of means (95% CI)	P-value (for trend)	Difference of means (95% CI)	P-value (for trend)
Vitamin C (mg)	1	0	0.26	0	0.44
	2	-0.002 (-0.01 to 0.01)		-0.0002 (-0.01 to 0.01)	
	3	-0.01 (-0.02 to -0.002)		-0.01 (-0.02 to -0.0005)	
	4	-0.003 (-0.01 to 0.004)		-0.002 (-0.01 to 0.01)	
	5	-0.005 (-0.01 to 0.004)		-0.003 (-0.01 to 0.01)	
Vitamin E (mg)	1	0	0.02	0	0.32
	2	0.001 (-0.01 to 0.01)		0.002 (-0.01 to 0.01)	
	3	-0.01 (-0.01 to 0.003)		-0.004 (-0.01 to 0.01)	
	4	-0.005 (-0.01 to 0.004)		-0.0002 (-0.01 to 0.01)	
	5	-0.01 (-0.02 to -0.004)		-0.01 (-0.02 to 0.01)	
Total vitamin A (µg)	1	0	0.15	0	0.19
	2	0.01 (-0.003 to 0.01)		0.01 (-0.002 to 0.01)	
	3	-0.005 (-0.01 to 0.004)		-0.004 (-0.01 to 0.004)	
	4	-0.005 (-0.01 to 0.004)		-0.004 (-0.01 to 0.005)	
	5	-0.002 (-0.01 to 0.01)		-0.001 (-0.01 to 0.01)	
Omega 3 fatty acids (mg)	1	0	0.83	0	0.52
	2	-0.003 (-0.01 to 0.01)		-0.003 (-0.01 to 0.01)	
	3	0.001 (-0.01 to 0.01)		0.002 (-0.01 to 0.01)	
	4	-0.001 (-0.01 to 0.01)		0.002 (-0.01 to 0.01)	
	5	-0.002 (-0.01 to 0.01)		0.001(-0.01 to 0.01)	
Ratio n6/n3	1	0	0.54	0	0.69
	2	-0.002 (-0.01 to 0.01)		-0.001 (-0.01 to 0.01)	
	3	-0.01 (-0.02 to 0.01)		-0.01 (-0.01 to 0.002)	
	4	0.002 (-0.01 to 0.01)		0.002 (-0.01 to 0.01)	
	5	-0.01 (-0.01 to 0.004)		-0.003 (-0.01 to 0.01)	
Selenium (µg)	1	0	<0.001	0	0.09
	2	-0.002 (-0.01 to 0.01)		-0.003 (-0.01 to 0.01)	
	3	-0.004 (-0.01 to 0.005)		-0.001 (-0.01 to 0.01)	
	4	-0.01 (-0.02 to -0.003)		-0.01 (-0.02 to 0.003)	
	5	-0.02 (-0.03 to -0.01)		-0.01 (-0.03 to 0.0001)	
Zinc (mg)	1	0	<0.001	0	0.14
	2	0.005 (-0.004 to 0.01)		0.01 (-0.003 to 0.01)	
	3	-0.005 (-0.01 to 0.003)		-0.002 (-0.01 to 0.01)	
	4	-0.02 (-0.03 to -0.01)		-0.01 (-0.02 to 0.0001)	
	5	-0.01 (-0.02 to -0.002)		-0.001 (-0.01 to 0.01)	

Table 8.7: Association between ratio FEV_1/FVC and flavonoid intake

Flavonoids	Quintile groups	Unadjusted model		Adjusted model	
		Difference of means (95% CI)	P-value (for trend)	Difference of means (95% CI)	P-value (for trend)
Flavones (mg/d)	1	0	0.88	0	0.77
	2	-0.001 (-0.01 to 0.01)		-0.001 (-0.01 to 0.01)	
	3	0.005 (-0.004 to 0.01)		0.002 (-0.01 to 0.01)	
	4	-0.001 (-0.01 to 0.01)		-0.002 (-0.01 to 0.01)	
	5	0.001 (-0.01 to 0.01)		-0.001 (-0.01 to 0.01)	
Flavonols (mg/d)	1	0	0.18	0	0.99
	2	-0.001 (-0.01 to 0.01)		0.001 (-0.01 to 0.01)	
	3	-0.003 (-0.01 to 0.01)		-0.001 (-0.01 to 0.01)	
	4	-0.01 (-0.01 to 0.004)		-0.002 (-0.01 to 0.01)	
	5	-0.004 (-0.01 to 0.004)		0.002 (-0.01 to 0.01)	
Total catechins (mg/d)	1	0	0.001	0	0.09
	2	-0.002 (-0.01 to 0.01)		0.0003 (-0.01 to 0.01)	
	3	-0.01 (-0.02 to -0.002)		-0.01 (-0.02 to 0.003)	
	4	-0.01 (-0.02 to -0.004)		-0.01 (-0.02 to 0.002)	
	5	-0.01 (-0.02 to -0.003)		-0.01 (-0.01 to 0.004)	

Table 8.8: Association between ratio FEV_1/FVC and plasma levels of biomarkers

Plasma biomarker	Quintile groups	Unadjusted model		Adjusted model	
		Difference of means (95% CI)	P-value (for trend)	Difference of means (95% CI)	P-value (for trend)
FRAP (μM)	1	0	0.16	0	0.95
	2	0.003 (-0.01 to 0.02)		0.01 (-0.01 to 0.02)	
	3	-0.02 (-0.03 to -0.003)		-0.01 (-0.02 to 0.0002)	
	4	0.001 (-0.01 to 0.01)		0.01 (-0.01 to 0.02)	
	5	-0.01 (-0.02 to 0.004)		-0.0002 (-0.01 to 0.01)	
Uric Acid (mg/dL)	1	0	0.27	0	0.77
	2	0.01 (-0.005 to 0.02)		0.01 (-0.003 to 0.02)	
	3	0.003 (-0.01 to 0.02)		0.01 (-0.005 to 0.02)	
	4	-0.005 (-0.02 to 0.01)		0.002 (-0.01 to 0.01)	
	5	-0.001 (-0.01 to 0.01)		0.01 (-0.01 to 0.02)	
Protein Carbonyls (nm/mg protein)	1	0	0.12	0	0.26
	2	-0.004 (-0.02 to 0.01)		-0.01 (-0.02 to 0.01)	
	3	-0.01 (-0.02 to 0.004)		-0.01 (-0.02 to 0.003)	
	4	-0.01 (-0.02 to 0.01)		-0.01 (-0.02 to 0.01)	
	5	-0.01 (-0.02 to 0.003)		-0.01 (-0.02 to 0.004)	
F2-isoprostanes (pg/mL)	1	0	0.89	0	0.56
	2	0.01 (0.02 to 0.03)		0.02 (0.01 to 0.03)	
	3	0.01 (-0.01 to 0.02)		0.01 (-0.001 to 0.02)	
	4	-0.001 (-0.01 to 0.01)		0.003 (-0.0001 to 0.02)	
	5	0.01 (-0.004 to 0.02)		0.01 (-0.0001 to 0.02)	

CHAPTER 9
Discussion

9.1 MAIN FINDINGS, STRENGTHS AND LIMITATIONS OF THE THESIS

9.1.1 Main findings

Overall, there were a large number of tests carried out that showed none or little evidence for an association between dietary intake of antioxidants and fatty acids, and biomarkers of oxidative stress with respiratory outcomes. An exception was the negative association found between BHR slope and intake of vitamin C, which was observed univariately and in the multivariable models. As Bonferroni correction gave a P-value above 0.05 this may be a type I error due to the number of tests carried out. Contrary to that expected, BHR slope was positively associated with intake of selenium, with a P-value below 0.05 after Bonferroni correction. In addition, a greater FEV_1 was positively associated total intake of catechins and plasma levels of FRAP were positively associated with FEV_1.

9.1.2 Strengths of the study

This thesis is the first population-based study carried out in a Latin American country assessing the association between dietary intake and several biomarkers of oxidative stress with lung function, respiratory symptoms, atopy and BHR, in a sample comprising nearly 1,200 young adults. This is also the first study assessing the association between the biomarkers F2-ip, protein carbonyls, uric acid and FRAP with respiratory outcomes related to asthma in a community-based study. The limited evidence on F2-ip and protein carbonyls as biomarkers of oxidative stress in asthma comes largely from clinical studies that have included a small number of participants with specific conditions of asthma and may not reflect the situation in the general population.

The prevalence of respiratory symptoms and the assessment of lung function, and atopy were determined using a standardised methodology based on the protocol of the ECRHS, which was aimed to estimate the prevalence of asthma and its risk factors across Europe. The use of such a protocol has the advantage that it allows comparison

between the prevalence of asthma in Chile and that in Europe and to explore the aetiology of the disease using the same instruments.

Another strength of this study was that trained professionals administered the questionnaires to collect information on diet, asthma and risk factors. This reduced the possibility of having incomplete questionnaires.

The FFQ used in this study was specifically designed to assess consumption of dietary antioxidants and fatty acids, and those food items considered part of the usual dietary intake of the people in Chile, thus including a selection of 62 food items. The professionals who administered the questionnaire were unaware of the antioxidant hypothesis being tested, thus diminishing the risk of information bias.

Most of the results published so far on asthma and diet have the limitation of focusing on particular nutrients or food items in isolation. This thesis examined a wide range of foods and nutrients. The study of Woods and colleagues is the only one so far looking at the associations of food and nutrient intake with asthma [106], which included self-reported symptoms of asthma, and measurements such as BHR and atopy. In spite of analysing a wide range of individual nutrients, only a few grouped food items were presented in the study of Woods *et al.* Information in relation to fruits and vegetables was limited, with three categories for fruits (1-2 pieces a day, apples and pears together, and berries), and vegetables (2-4 servings, green leafy, and tomatoes) so it provided limited range of sources of antioxidants studied. In addition, they adjusted for very few potential confounders, which for example did not include socio-economic level, an important determinant of dietary intake and asthma.

In the current study the association between dietary intake and asthma was explored not only in relation to self-reported symptoms of asthma but also to BHR, a more objective measurement, in contrast to most of the current epidemiological evidence limited to self-reported symptoms [332]. This may have been an advantage, as it reduced possible cultural bias in people that may for example, misunderstand the concept of wheeze or interpret it as something different.

The choice of using the tidal breathing method to assess BHR facilitated the test in the participants as it requires breathing normally during two minutes instead of matching the exhalation with the exact time at which the methacholine is released. In Chile, the tidal breathing method has been commonly used in the specialised services, and the nurses received training in a hospital dedicated to respiratory diseases in order to be able to perform the manoeuvre correctly.

9.1.3 Limitations of the study

The design of this study was cross-sectional, and so it is not possible to establish the temporality of the association between nutrient or food exposure and respiratory outcomes. The endpoints of interest were present at the time when data were collected, and thus an assessment of disease status and exposure were at a single point in time, making it unfeasible to determine whether the exposure preceded or resulted from the disease.

Another limitation of this thesis is related to the use of an FFQ to estimate dietary intake. It is well known that assessing nutrient intake through questionnaires may introduce measurement and recall bias. In this study it is unlikely that dietary reporting bias had occurred, as all the participants were interviewed about many other aspects of health than dietary intake, and were unaware of any of the hypotheses being tested, in particular about the link between antioxidant nutrients and asthma. The FFQ in this study was standardised, based on a pilot study first, and compared to a 24 hour-recall questionnaire. The validation study showed that the level of agreement was good to excellent for most of the food items, with the exception of fish, whose consumption was very low by either questionnaire but reported higher in the FFQ than in the 24-hours recall.

A third limitation of the study was that the assessment of biomarkers of oxidative stress and of antioxidant status was only possible in approximately 50% of the participants. This occurred because the study had already started when funding for determination of these biomarkers was obtained and it was unfeasible to take again blood samples to

estimate these biomarkers in a proportion of the participants. Nevertheless, this is the largest epidemiological study assessing F2-ip in general population.

In addition it was not possible to assess biomarkers of nutritional exposure because of the lack of facilities to assess them in Chile, which prevented this study from contributing to the current debate on the effect of plasma levels of vitamins and minerals, for example, in relation to symptoms of asthma. The measurement of FRAP contributed to overcoming this limitation, as it provides an indirect estimation for activity of vitamin C and flavonoids in plasma.

The assessment of biomarkers of nutritional exposure such as vitamin C and others in different tissues and fluids can be considered a more reliable or at least less subjective estimation of nutrient exposure, although is not free from limitations related to reproducibility, validation and interpretation of causality [333]. This may partly explain the inconclusive epidemiological evidence obtained from dietary intake in relation to asthma symptoms as reviewed in Chapter 3.

All the biomarkers of this study were assessed in plasma, and may not reflect oxidative stress as direct consequence of asthma as would other biomarkers assessed in more specific sites. FRAP and uric acid are indicators of the general antioxidant capacity observed in plasma, while carbonyls of proteins reflect general oxidative damage. F2-ip is a marker and a mediator of oxidative damage, and its production is related to the oxidation of specific fatty acids involved in the inflammatory response implicated in the asthmatic response, which adds more precision to the possible oxidative imbalance derived from asthma. It may be suggested that assessment of these biomarkers in more specific sites such as exhaled breath or condensate air may ascertain better the oxidative stress related to asthma, but the current evidence is still very limited to draw such conclusions.

9.2 JUSTIFICATION IN THE USE OF SELECTED CONFOUNDERS

In this study very few antioxidants showed an association with any of the outcomes studied before and after controlling for confounders. One of the factors affecting the estimation of dietary intake from an FFQ is the heterogeneity of the population studied

in terms of age and sex, but these considerations did not affect the lack of associations in the majority of the analyses. With BHR and respiratory symptoms, for example, little change was observed when adjustments for potential confounders were carried out.

As many comparisons were carried out in this study, the issue of whether further adjustments to P-values were necessary was considered. An adjustment for multiple comparisons in epidemiological studies has been considered unnecessary by some [334, 335], essential by others [336], or subject to the investigator's decision [337]. In this study, the Bonferroni correction was carried out in those tests when a nominal statistically significant association was found. Thus, a conservative approach was taken.

9.2.1 Adjustment for TEI

The effect of TEI was assessed in a separate model with all the other potential confounders (Appendices 2 and 3) in order to assess the effect of measurement error. The FFQ designed for this study, ascertained intake of individual food items and nutrients in order to be more specific of which food items may or may not account for associations, but the inclusion of many food items tends to overestimate TEI [338]. In the design of this FFQ, every attempt was made to cover the most traditional food items consumed by Chileans and also those that were important for their content of antioxidants or fatty acids. Taking into account this criterion, the FFQ was limited to just 62 food items. In spite of these precautions, overestimation of macronutrients was confirmed in the validation carried out against a 24 hours questionnaire administered on three occasions to a sample of the participants, which justifies further the adjustment for energy intake.

As suggested by Willet, measurement errors for specific nutrients tend to be highly correlated with errors for total energy intake, because they are calculated from the same food items. Thus, adjustment for TEI will control for the effect of correlation between nutrients and TEI [339]. Macronutrients are usually highly correlated with TEI, and lack of adjustment by energy intake when associations with nutrients are

sought can obscure true associations –or the lack of them- or provide spurious associations between nutrient intakes and disease [339].

A constraint in several observational studies is that TEI is not adjusted for when assessing relations between diet and respiratory outcomes, and it is possible that these findings or the lack of them may be obscured by the effect of TEI. For example, some authors have reported a beneficial inverse association between intake of vitamin C and morning cough [107], BHR [111], atopy [122], and allergic asthma [113], but none of these were adjusted for TEI.

A number of other studies that have controlled for a limited number of potential confounders but excluded TEI, have reported a beneficial inverse association between consumption of vegetables and bronchial asthma [340], between intake of green leafy vegetables and current asthma [106], or tomatoes and atopy [106]. These findings could be obscured by the effect of TEI. The same limitation arises from studies that have found positive associations between fruit intake and a greater FEV_1 [158], incidence of asthma [154], current asthma, BHR and atopy [106].

In the case of asthma and BMI, there has been much debate on whether body size and obesity play a role in the incidence of the disease or if they are concurrent diseases [341]. This hypothesis was tested in the population of Limache, finding that BMI was positively associated with symptoms of asthma but it was negatively associated with BHR. Waist circumference was unrelated to asthma symptoms and BHR, thus suggesting that a causal relationship between body size and asthma is unlikely [342].

9.2.2 Adjustment for socio-economic and demographic variables

As reviewed by Tohill *et al.*, epidemiologic studies indicate that several socio-economic and demographic factors that could be related to a disease are also strongly related to consumption of fruits and vegetables [343]. In this thesis, the analyses of fruits and vegetables included adjustments for sex, age, and height (in the case of FEV_1), and a number of socio-economic and demographic variables. There is sound evidence that a higher consumption of fruits and vegetables has been associated with being older, higher educational level, being less sedentary, not smoking, as well as a

general healthier diet characterised by lower consumption of fats and of red meat [344-348].

A methodological limitation in several observational studies is the lack of adjustment for socio-economic variables or educational level, when assessing the relation between consumption of fruits and vegetables and respiratory symptoms or lung function. The large cross-sectional study by Tabak *et al.* in adults from Finland, Italy and The Netherlands reported a positive association between FEV_1 and fruit in those with an intake above the median, but adjustments for socio-economic factors or educational level were not made [97]. Similarly, in the MORGEN study a beneficial association was reported on intake of fruit juice and vegetables with FEV_1, but the authors did not adjust for socio-economic level [155]. Knekt and colleagues reported a beneficial association between apple and orange intake with incidence of asthma in a retrospective longitudinal study, but with a limited adjustment for confounders [154].

9.3 MAIN ASSOCIATIONS BETWEEN ANTIOXIDANTS AND RESPIRATORY OUTCOMES

9.3.1 Associations between antioxidant vitamins and measurements of asthma

Vitamins C, E and A are the nutrients most extensively investigated in epidemiologic studies for their antioxidant and in the case of vitamins C and E, anti-inflammatory and anti-allergic effects on asthma. In this study, the daily consumption of all these vitamins was well above the EAR and also higher than the recommendations given by the Chilean Ministry of Health. Intake of vitamin E was almost 5 times higher than recommended values, while those of vitamin C and A were triple and double the recommendation, respectively.

A negative association was found between intake of vitamin C and BHR slope. The multivariable analyses with BHR slope controlled for FEV_1%/FVC, FEV_1% and atopy as potential confounders, so this finding was not explained by lung function or skin allergen sensitisation variation. The fact that the intake observed in this population was well above the RNI may lend further support to this possible protective effect, on the grounds that vitamin C is a powerful antioxidant and its modulator effect on the

respiratory tract can be observed with higher intakes of the antioxidant when provided with the diet.

It could be argued that this is a chance finding, as although it remained statistically significantly associated with BHR slope after controlling for all potential confounders, the P-value was no longer significant after Bonferroni correction. It is expected that the tests could produce some type I errors due to the multiple comparisons carried out.

The evidence from other observational studies is very limited in relation to vitamin C and BHR to draw conclusions on the possible effects of this antioxidant. A cross-sectional study carried out in a sample of 1,601 Australian adults found no association between a per mg increase in intake of vitamin C and BHR (OR 0.96, CI: 0.71 to 1.31) [106], while in a small case-control study, Soutar and colleagues showed that those with a positive response to methacholine had significantly lower intake of vitamin C than those who did not have a positive reaction [111].

In the current study, there was no association between respiratory symptoms, or BHR and increasing intake of vitamin E. This is in agreement with the large majority of evidence currently available from observational studies that have assessed intake of vitamin E in relation to wheeze [94, 109], asthma [110], seasonal symptoms (allergy, atopy, eczema) [111], cough [94], phlegm [94], shortness of breath [94], current asthma [106], asthma ever [106], and BHR [111].

There is some evidence from a longitudinal study that high vitamin E intake is associated with a lower incidence of asthma over a ten-year period in women [114], and that plasma levels of vitamin E are lower in adults with moderate allergic asthma (as defined by GINA) when compared to healthy individuals [123]. In contrast, one study reported that those with a higher intake of vitamin E had a higher prevalence of productive cough [94]. The lack of consistent findings indicates that a high degree of uncertainty over the effect of vitamin E on asthma remains.

9.3.2 Association between antioxidant vitamins and lung function

Dietary intake of vitamins or vegetables was unrelated to measurements of lung function in the adults from Limache. The epidemiological evidence on diet and respiratory health is suggestive of some beneficial effect of vitamin C on lung function as reviewed in detail in Chapter 3. This thesis does not provide evidence to support these findings.

Similarly, vitamin E in adults from Limache was not associated with lung function. In this regard, the epidemiological evidence is far less conclusive than that for vitamin C and it will require further studies to clarify its role if any. Britton and colleagues reported an increase of 20.1 ml and of 5.3 ml for FEV_1 and FVC in adults from Nottingham as the intake of vitamin E increased by 1 SD [92]. Butland *et al.* also showed that a 1 SD increase of vitamin E was associated with a 39.1 ml increase of FEV_1 after, but not before adjusting for energy intake, and a weaker but still significant increase remained after controlling for vitamin C and apple intake [100].

Hu and colleagues found a significant increase in FEV_1 in their study when vitamin E was the only nutrient in the multivariable model, but this beneficial association was no longer observed when vitamin C and β–carotene were included simultaneously with vitamin E [96]. Two other large cross-sectionals studies found no association between intake of vitamin E and FEV_1 [94, 97] or FVC [94].

9.3.3 Association between vegetable or fruit intake and respiratory symptoms, BHR or lung function

The main analyses of this thesis showed no association between intake of fruits and vegetables with any of the outcomes studied. It has to be pointed out that the additional statistical analyses carried out with individual food items showed statistically significant associations, although the P-values were raised above 0.1 after Bonferroni correction (Appendices 2 and 3).

A positive association was observed between intake of garlic and onion and a greater FEV_1, (Appendix 3, Table E (1)) as well as a negative association with BHR slope (Appendix 2, Table A (1)). After Bonferroni correction these associations lost statistical significance. It is known that garlic and onions are rich in organosulfur compounds [349]. Additionally, onions are characterised by their high content of flavonoids, particularly anthocyanins and flavonols (such as quercetin and its derivatives).

The organosulfur compounds, specifically the alk(en)yl cysteine sulphoxides found in these two foods, act as antioxidants with free radical–scavenging properties to inhibit lipid peroxidation [350]. The flavonoids found in onions can function as chain-breaking antioxidants by scavenging some radical species [351]. They may suppress lipid peroxidation by recycling other antioxidants, such as α-tocopherol, by donating a hydrogen atom to the α-tocopherol molecule. In addition, they can chelate pro-oxidant metal ions, such as iron and copper, thus preventing free radical formation from these pro-oxidants while simultaneously retaining their own free-radical scavenging capability [351].

These facts may contribute to prevent or diminish the damage induced by oxidative by irritants such as the methacholine, and facilitate a general better maintenance of the oxidant balance in the lungs. Although a possible chance finding cannot be ruled out, the potential public health significance of these findings should be explored further.

Regarding legumes, it was observed that consumption of lentils was related to a lower risk of prevalence of having at least one respiratory symptom, which was statistically significant before but not after Bonferroni correction (Appendix 2, Table D (1)). Most of the evidence in relation to intake of legumes and asthma is about the glycoproteins that they contain, which have been recognised as food allergens [352, 353]. However, it has been recognised that legumes, and in particular lentils, are rich in proanthocyanidins, the major group of polyphenols, and also have some content of catechins, such as (+) catechin-3-glucose, (+)-catechin and (-)-epicatechin [354]. The findings of the negative association observed in the current study between lentils and respiratory symptoms could be explained by the antioxidant properties displayed by

these antioxidants, and lend further support to the hypothesis that polyphenols and flavonoids could have a beneficial role against respiratory symptoms.

9.4 INTAKE OF FLAVONOIDS

In the current study the relation between symptoms of asthma and BHR, as well as lung function measurements, with intake of three major subclasses of flavonoids was assessed. There was a predominant lack of associations found in these analyses with the exception of an association between total catechins intake and best FEV_1. These findings are in line with Tabak *et al.* who found that total intake of catechins were positively associated with a greater FEV_1 in a sample of 17,453 adults from The Netherlands [155].

There is limited epidemiological evidence of an association between different classes of flavonoids and respiratory symptoms. Very recently we have found no association between any of the three classes of flavonoids and asthma or chronic sputum in adults from London [156]. Another study reported that quercetin (the main flavonol), and herperitin and naringenin (flavonones), but not catechins, were negatively associated with incident asthma [154]. In the current study, no associations were found for flavonols and flavones and symptoms of asthma, and although flavonones were not measured, none of their dietary sources (orange, lemon and kiwi) were related to any respiratory symptom or BHR.

The median (IQR) intake of total flavonoids in the Chilean population was 48.7mg (24.2 to 113.5). A mean intake of 58 mg/d (SD 46) was reported for a study of adults in The Netherlands, which assessed the same classes of flavonoids as the current study [155]. A cohort study of 10,040 Finnish adults reported lower values of total flavonoid intake. As in this study, they assessed flavones (quercitin, kaempferol and myricetin) and a subclass of flavonones (naringenin and hesperitin) that were not calculated in the Chilean population, but excluded measurements of catechins, which may explain the differences in the observed intakes [154].

The median (IQR) intake of catechins in Chileans was 19.1 mg/day (8.8 to 66.4), similar to that reported in postmenopausal American women [355], and considerably

lower than that reported by us in adults from London (median (IQR) 81.2 (32.5 to 135.7) mg/day) [156] and by others in Dutch elderly men [356]. The intake of total catechins in the studied adults agrees with the average one cup of tea a day reported.

The FFQ administered in Chile was specifically designed to ascertain consumption of flavonoid-rich food items, taking into account their availability in the area where the study was conducted and their being part of the usual dietary intake of this population. These included apple, strawberry, garlic, onion, tea and red wine. The list of foods included in the questionnaire was revised and agreed by the Dutch creators of the database that collaborated with the study (Arts, personal communication).

Additional individual analyses with tea, apples and red wine (main dietary sources of catechins), demonstrated no associations with the primary outcomes of interest or with measurements of lung function in this study, which could indicate that the beneficial effect of catechins may not be causal. It has to be noted that the two main catechins found in tea, namely epigallocatechin gallate (ECGg) and epicatechine gallate, are less biologically active *in vivo* than are other catechins [357].

Flavonoids have the capacity to reduce asthma inflammation through antioxidant, anti-allergic and anti-inflammatory properties by different mechanisms, which include their ability to act as scavengers of nitric oxide, inhibiting histamine release, arachidonic acid metabolism, and cytokine production [351].

A positive association between a higher intake of catechins and having at least one respiratory symptom was also found. One possibility is that despite their well-known antioxidant activity, the pro-oxidant effect previously mentioned for EGCg may have explained these results.

9.5 Intake of fatty acids

9.5.1 Omega 6 and omega 3 fatty acids

The estimated 47:1 ratio n6/n3 found in this population was much higher than the range recommended as healthy. According to Simopolous, the healthy range varies

from 1:1 to 5:1 depending on the disease under consideration [358], with a general recommendation of 2:1 [329]. Such a high ratio in this population is explained by the low consumption of fish, which only reached 10g per day in average, in line with other studies on dietary habits in the country [359] and with the per capita intake of 5.1kg/year described for the Chilean population [166]. This ratio also corresponds to the increased consumption of omega 6 found in hydrogenated oils. This is representative of the current tendency observed in Chile [166, 359], characterised by a steady rise in the consumption of products manufactured with hydrogenated oils, and a decrease in the consumption of solid fats of animal origin, including butter.

In the analyses, there was no indication of an association between intake of fatty acids omega 3 and respiratory symptoms of asthma, BHR or lung function. There is evidence that dietary supplementation with omega 3 reduces AA concentration in neutrophils, generation of LT [360] and airway late response to allergen exposure [188], which is consistent with the proposed mechanisms by which omega 3 modulates asthma and inflammation in the respiratory tract [167]. The findings in this Chilean population are not suggestive of such a beneficial effect, at least for asthma.

9.6 Biomarkers of oxidative stress and antioxidant status

9.6.1 F2-ip

Levels of oxidative stress as assessed from plasma F2-ip did not show any association with the measurements of asthma, with one exception. A nominal statistically significant negative association was observed between plasma F2-ip and having at least one respiratory symptom in the adjusted model, but the Bonferroni correction raised the P-value above 0.1.

The measurement of plasma levels of F2-ip in this population was undertaken for several reasons. Firstly, since its discovery, increasing clinical and experimental evidence has demonstrated that the level of F2-ip is a good indicator of oxidation *in vivo* [38]. Secondly, the production of F2-ip has relevance in relation to asthma, as its production is derived from AA, and this PUFA is directly associated with the inflammatory events that take place during the manifestation of asthma symptoms.

Therefore it would be expected that higher levels of F2-ip would be found with higher prevalence of manifestations of inflammation.

In addition, F2-ip is relevant for the study of asthma as it is not only a biological marker but also a mediator of oxidative stress, and thus may play an active causative role in the development of the disease. A number of experimental studies have demonstrated that F2-ip is a mediator of inflammation, jointly with other isoprostanes, directly involved in the pathology of asthma, and that it affects airway smooth muscle contraction, and it is a potent bronchoconstrictor [361-362].

The results of the current study do not support any of the findings of experimental evidence. One reason to be taken into consideration is that most of the existing data were obtained in rodents. There is evidence of different response to F2-ip according to the type of animal where the biomarker is assessed. For example, airways in rabbits [363] and dogs [364] do not constrict in response to either 8-iso-PGE_2 or F2-ip, and a relaxant response to F2-ip has been observed in the pulmonary arteries of rat [365]. Therefore, the strength of the evidence has to be viewed with caution. There are relatively few studies done in human tissues suggesting that is a constrictor agent [364, 366, 367] and from human pulmonary vessels demonstrating that F2-ip is released under conditions of oxidative stress when exposed to NO or COX [369], but these findings may not reflect what occurs in population-based studies.

F2-ip is produced in variable amounts both in conditions of health and illness, so it is normal to detect F2-ip, at least in plasma, in healthy subjects. In this study, the mean value of plasma F2-ip was 30pg/ml (range 5.6 to 95.4), which may be a reference for the range within which values of F2-ip may be found in the community.

This study assessed F2-ip in plasma, a less specific measure of oxidative stress than that of other sites. Plasma is much more likely to indicate normal synthesis that occurs in the cell membranes during processes of reactions of oxidation-reduction in different organs and systems and is not as specific as those of airways. A large proportion of F2-ip obtained from exhaled breath condensate comes from that produced and released from the respiratory airways, suggestive of production of lipid peroxidation occurring in the airways rather than mirroring any systematic process of oxidative stress.

However, this may not always be possible to assess in large community studies due to the high cost involved. In this study, the methodology to determine exhaled F2-ip or NO was not feasible due to its high cost at the time the study was carried out.

The methodology used for the estimation of F2-ip in this population was based in an immunoassay, which is one of the several techniques utilised for determination of PG-like compounds. As indicated by Roberts *et al.* [38], the main limitation of this technique is that interfering substances in biological fluids (i.e. plasma, serum) can be encountered and interfere with the immunoassay. As immunoassays can produce an accurate measure in buffer systems that do not contain large amounts of biological substances [38], more recent kits, such as the one used for the current study, include buffer solutions and purification to eliminate interfering substances, thus increasing the reliability of the test. In a recent review, Morrow highlighted that immunoassays are becoming increasingly used in quantification of F2-ip due to their low cost and relative ease of use [368].

The lack of association between F2-ip and most of the respiratory outcomes may also correspond to the fact that those who reported respiratory symptoms may have had a mild form of asthma rather than a more severe manifestation of symptoms. It may be possible that differences in F2-ip can be detected when more severe manifestations of symptoms are present, and thus, a much greater production of the biomarker will be produced in comparison to those with no symptoms at all.

Inclusion of BMI and sex as confounders was considered based on previous evidence suggesting that women are more likely to have higher values of F2-ip [203] and of lipid peroxides [369], possibly linked to a higher deposit of body fat, and thus of substrate for lipid peroxidation. However, in this study controlling by sex and BMI did not affect the relationship between F2-ip and lung function, BHR, or respiratory symptoms in the presence of atopy.

The finding of increased levels of F2-ip both in the circulation and in the urine of smokers [198, 370] is consistent with the fact that cigarette smoke contains a large number of oxidants and free radicals that could directly initiate and propagate the

process of lipid peroxidation [371]. In the present study, we controlled for smoking but even without adjustments did not find an association.

9.6.2 Protein carbonyls

Little has been reported regarding protein carbonyls and their association with asthma. As mentioned in Chapter 4, determination of protein carbonyls has been considered a useful tool to determine oxidative stress in several diseases, with very limited evidence of its use in asthma. For this study, the assessment of carbonyls of proteins was chosen because, after PUFA, proteins are the most vulnerable molecules for oxidation. It would therefore be expected that, if oxidative stress is present, a higher production of carbonyls would exist, mainly as consequence of the oxidation that amino acids residues undergo in the presence of O_2. In this study no associations were found between plasma carbonyls and self-reported symptoms of asthma or BHR, suggesting a lack of association with the disease.

A lack of association between plasma carbonyls and respiratory symptoms of asthma was also found in a case-control study carried out in 38 subjects with mild, moderate and severe bronchial asthma and 23 age-matched healthy controls [229]. It may be possible that because carbonyls were assessed in plasma in that study as well as in the current study, circulating carbonyls in plasma corresponded to physiological amounts and not necessarily related to oxidative stress but to normal oxidation in the plasma. Possibly, carbonyls of proteins assessed in broncho-alveolar lavage [245] are specific of oxidation related to respiratory oxidation, although this has not been confirmed in carbonyls measured in sputum supernatant [246]. The evidence for this is limited to studies based on a small number of people with a severe asthma, and this methodology cannot be used in epidemiological studies.

Overall, it was not possible to confirm other findings of higher F2-ip or carbonyls of proteins in subjects with respiratory symptoms. The available evidence from clinical experiments should be regarded with caution, as they may not be applicable to population-based studies.

9.6.3 FRAP and uric acid

In this population, there were no associations between measurements of asthma and these biomarkers. The assessment of FRAP was chosen for two reasons. Firstly, it provides some insights on the potential action that antioxidants contained in the diet could exert, and this was particularly important due to inability to estimate plasma levels of vitamins in this study. FRAP is an assay that mirrors the antioxidant action of several compounds that contribute to the total antioxidant power of the plasma. These are ascorbic acid (15%), alpha-tocopherol (5%), bilirubin (5%), uric acid being the largest fraction of FRAP with an estimated contribution of 60% [262]. Secondly, it has been suggested the possible participation of polyphenols can also be estimated [263] due to their capacity to act as iron chelators, which can be detected with the measurement of FRAP.

The lack of associations found between biomarkers of oxidative stress and respiratory symptoms of asthma and BHR slope might suggest that F2-ip and protein carbonyls are not sufficiently specific in plasma or that their utility is reduced if assessed in general population, where more severe respiratory illnesses are scarce.

9.7 Conclusions and further directions

In this cross-sectional study, we found little supportive evidence of a relationship between dietary antioxidant intake and asthma, as assessed with subjective measurements of self-reported respiratory symptoms, or a more objective measure. There was a consistent pattern of lack of associations between antioxidant vitamins, minerals and food items with these primary outcomes.

The lack of associations found between different plasma biomarkers and respiratory outcomes of asthma is an important contribution to the current knowledge in this area. In spite of the relatively good experimental and clinical evidence for showing a positive association between F2-ip and asthma, this relation may not be found in community-based studies. This study provides a reference on the physiological range that can be expected in young adults.

A future study would benefit from the inclusion of oxidative stress assessed in more specific sites. The fact that FRAP was mostly unaltered in this population, confirms that there is a good balance between substances being oxidised and those reduced in plasma. The ranges found here were within the values reported as normal in the literature, and applied to a much higher number of people. The levels of markers of oxidative stress in plasma may not be related to asthma if the disease is not present with more severe symptoms or exacerbations. Therefore, new methodologies such as assessment in exhaled breath, or condensate air concentration of F2-ip or other more specific markers of oxidative stress like NO and MDA could provide a better insight on the relationship between general antioxidant status and susceptibility to oxidative stress in those that have asthma in the community.

The fact that some food items may reduce the risk of having BHR, as found with garlic and onion, raises the question of whether such effect is persistent. On the other hand, the issue of whether fruits and vegetables rich in antioxidants and the individual antioxidants have a real effect on the prevalence of asthma in adults requires further investigation.

This thesis provides limited evidence for associations between dietary intake of antioxidants and symptoms of asthma or lung function. The use of biomarkers of oxidative stress and antioxidant status in plasma in the general population may not be useful in reflecting oxidative damage related to asthma. It could be possible that a beneficial effect could be observed in populations exposed to lower levels of antioxidants than those in this study.

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APPENDIX 1
Questionnaires used in the thesis

This appendix includes two parts: (A) A translated version of the ECRHS questionnaire administered to the participants of the study; (B) the Lung Function Questionnaire (Part 1) and Data Collection Sheet (Part 2); and (C) a translated version of the FFQ.

The translated ECRHS is slightly different to that used for the European study, where several questions were aimed to assess the use of medication in these adults. For the purposes of the study in Chile, it was assumed that very few individuals would have received medication. This information was obtained in a shorter way.

There were a number of other items added in order to adapt the questionnaire to the local situation of Limache, especially those related to socio-economic background.

A: ENGLISH TRANSLATION OF THE MAIN QUESTIONNAIRE USED IN LIMACHE

Area number

Personal number

Sample

Date DAY MONTH YEAR

I AM GOING TO ASK YOU SOME QUESTIONS. AT FIRST THESE WILL BE MOSTLY ABOUT YOUR BREATHING. WHEREVER POSSIBLE, I WOULD LIKE YOU TO ANSWER 'YES' OR 'NO'.

Wheeze and tightness in the chest

1. Have you had wheezing or whistling in your chest at any time in the last ***12 months***? NO YES

IF 'NO' GO TO QUESTION 2, IF 'YES':

1.1 Have you been at all breathless when the wheezing noise was present? NO YES

1.2. Have you had this wheezing or whistling when you did *not* have a cold? NO YES

2. Have you woken up with a feeling of tightness in your chest at any time in the last ***12 months***? NO YES

Shortness of breath

3. Have you had an attack of shortness of breath that came on during the day when you were at rest at any time in the last ***12 months***? NO YES

4. Have you had an attack of shortness of breath that came on ***following*** strenuous activity at any time in the last ***12 months***? NO YES

5. Have you been woken by an attack of shortness of breath at any time in the last ***12 months***? NO YES

Cough and phlegm from the chest

6. Have you been woken by an attack of coughing at any time in the last ***12 months***? NO YES

6.1 Was this cough related to a cold at that time? NO YES

7. Do you ***usually*** cough first thing in the morning in the winter? NO YES

[IF DOUBTFUL, USE QUESTION 8.1 TO CONFIRM]

8. Do you ***usually*** cough during the day, or at night, in the winter? NO YES

IF 'NO' GO TO QUESTION 9, IF 'YES':

8.1 Do you cough like this on most days for as much as three months each year? NO ☐ YES ☐

9. Do you ***usually*** bring up any phlegm from your chest first thing in the morning in the winter? **[IF DOUBTFUL, USE QUESTION 10.1 TO CONFIRM]** NO ☐ YES ☐

10. Do you ***usually*** bring up any phlegm from your chest during the day, or at night, in the winter? NO ☐ YES ☐

IF 'NO' GO TO QUESTION 11, IF 'YES':

10.1 Do you bring up phlegm like this on most days for as much as three months each year? NO ☐ YES ☐

<u>Breathing</u>

11. Do you ever have trouble with your breathing? NO ☐ YES ☐

IF 'NO' GO TO QUESTION 12, IF 'YES':

11.1 Do you have this trouble

TICK ONE BOX ONLY

a) continuously so that your breathing is never quite right? ☐
b) repeatedly, but it always gets completely better? ☐
c) only rarely? ☐

12. Are you disabled from walking by a condition ***other than*** heart or lung disease? NO ☐ YES ☐

12.1 If yes, state condition: _____

12.2 Are you troubled by shortness of breath when hurrying on level ground or walking up a slight hill? NO ☐ YES ☐

IF 'NO' GO TO QUESTION 13, IF 'YES':

12.2.1 Do you get short of breath walking with other people of your own age on level ground? NO ☐ YES ☐

12.2.2 Do you have to stop for breath when walking at your own pace on level ground? NO ☐ YES ☐

13. <u>Only for women</u> and only if you have problems with your breathing.
Do your breathing problems (wheezing, chest tightness, difficulty to breath) happen in a particular moment of your menstrual period?

TICK ONE BOX ONLY

13.1 Yes, during the week previous to the start of my period ☐
13.2 Yes, during my period ☐

13.3 Yes, in the week after my period ☐
13.4 Yes, in another moment of the month ☐
13.5 No, because I do not have periods ☐
13.6 No, because I am a male ☐
13.6 No, it has nothing to do with my periods ☐

Asthma

NO YES

14. Have you ever had asthma? ☐ ☐

IF 'NO' GO TO QUESTION 15, IF 'YES':

NO YES

14.1 Was this confirmed by a doctor? ☐ ☐

YEARS

14.2 How old were you when you had your first attack of asthma? ☐☐

YEARS

14.3 How old were you when you had your most recent attack of asthma? ☐☐

14.4.1-6 Which months of the year do you usually have attacks of asthma?

NO YES

14.4.1 January / February ☐ ☐
14.4.2 March / April ☐ ☐
14.4.3 May / June ☐ ☐
14.4.4 July / August ☐ ☐
14.4.5 September / October ☐ ☐
14.4.6 November / December ☐ ☐

NO YES

14.5 Have you had an attack of asthma in the last ***12 months***? ☐ ☐

IF 'NO' GO TO QUESTION 15, IF 'YES':

NUMBER

14.6 How many attacks of asthma have you had in the last ***12 months***? ☐☐

NUMBER

14.7 How many attacks of asthma have you had in the last 3 ***months***? ☐☐

NUMBER

14.8 How many times have you woken up because of asthma in the last 3 months?

14.8.1 Nearly every night ☐☐
14.8.2 More than once a week ☐☐
14.8.3 More than once a month but less than once a week ☐☐
14.8.4 Less than twice a month ☐☐
14.8.5 None ☐☐

14.8.6 I have not had this problem because I take medicines to avoid it ☐☐

14.9 How often has asthma caused you problems in the last 3 months? NUMBER

14.9.1 Frequently ☐☐

14.9.2 Once a day ☐☐

14.9.3 More than once a week but less than once a day ☐☐

14.9.4 Less than once a week ☐☐

14.9.5 None ☐☐

14.9.6 I have not had this problem because I take medicines to avoid it ☐☐

14.10 Has a doctor given you written instruction on how to manage your asthma, or what to do if you have an asthma attack? NO ☐ YES ☐

<u>Allergies</u>

15. Do you have any nasal allergies, including hay fever? NO ☐ YES ☐

15. 1 How old were you the first time you had this allergy? AGE ☐☐

16. Have you ever had a nasal secretion, or your nose blocked due to a congestion without having a cold? NO ☐ YES ☐

IF 'NO' GO TO QUESTION 17, IF 'YES':

16.1. Have you ever had a nasal secretion, or your nose blocked due to a congestion without having a cold in the **last 12 months**? NO ☐ YES ☐

IF 'NO' GO TO QUESTION 17, IF 'YES':

16.1.1 When this happens, do you also have red eyes and itchiness? NO ☐ YES ☐

16.1.2 In what time of the year does this nasal problem occurs?

a) January/February ☐
b) March/April ☐
c) May/June ☐
d) June/July ☐
e) September/October ☐
f) November/ December ☐

NO YES

17. Have you ever had eczema or any other type of allergy in your skin? ☐ ☐

18. Have you had redness or rash in your skin, appearing and disappearing in the last 12 months?

NO YES

☐ ☐

18. Which part of your body is affected by this rash and itchiness?

NO YES

19. Are you allergic to insect bites? ☐ ☐

19. 1. To what type of insects?

20. Have you ever had problems with your breathing after taking a medicine? NO YES ☐ ☐

20. 1. To what type of medicines?

NO YES

21. Are you allergic to any food? ☐ ☐

21. 1. To what type of food?

NO YES

22. Are you allergic to a plant or a tree? ☐ ☐

22. 1. To which plant or tree?

Your home

YEARS

23. How many years have you lived in your present home? ☐☐

YEARS

24. How many years have you lived in _____? ☐☐

25. When was your present home built?

TICK ONE BOX ONLY

a) before 1960? ☐
b) 1961-1970? ☐
c) 1971-1980? ☐
d) 1981 or later? ☐
e) don't know ☐

26. Which best describes the street where your house is?

TICK ONE BOX ONLY

a) a main street ☐
b) a secondary street ☐
c) a small street (mews, etc) ☐

27. Do cars pass in front of your house?

NO YES

a) Constantly
b) Frequently
c) Rarely
c) Never

28. Do heavy vehicles pass in front of your house?

NO YES

a) Frequently
b) Rarely
c) Never

29. Which of the following fuels do you use for heating or for hot water?

NO YES

29.1 open coal,
29.2 wood fire
29.3 gas
29.4 paraffin heater
29.5 electricity
29.6 other: _____

30. What kind of stove do you *mostly* use for cooking?

TICK ONE BOX ONLY

30.1 coal, coke or wood (solid fuel)?
30.2 gas?
30.3 electric?
30.4 paraffin?
30.5 other: _____

NO YES

31. Do you have an extractor fan over the cooker?

IF 'NO' OR 'DON'T KNOW' GO TO QUESTION 32, IF 'YES':

31.1 When cooking, do you use the fan

TICK ONE BOX ONLY

a) all of the time?
b) some of the time?
c) none of the time?

NO YES

31.2 Does the fan take the fumes outside the house?

32. Do you cook?

IF 'NO' OR 'DON'T KNOW' GO TO QUESTION 35, IF 'YES':

32.1 On average, how much time do you spend cooking every day?

MINUTES

33. In winter, is the door of the kitchen ever left open when cooking?

	NO	YES
33.1 All the time		
33.2 A few hours		
33.3 Rarely		
33.4 Never		

34. On which floor of the house is your bedroom?

---

35. Does your bedroom have upholstered or soft furnishings?

	NO	YES

36. Does the room where you spend most of your time in the house ?

	NO	YES
36.1 have fitted carpets covering the whole floor?		
36.2 contain rugs?		
36.3 have curtains?		
36.4 have upholstered or soft furnishings?		

37. If you have rugs, for how long have you had the oldest one in your house?:

	TICK ONE BOX ONLY
37.1 less than a year	
37.2 1-5 years	
37.3 more than 5 years	
37.4 don't have	

38. Of what material is your mattress?:

	NO	YES
38.1 Wool		
38.2 Sponge		
38.3 Other (specify)		

39. For how long have you had your mattress?

	TICK ONE BOX ONLY
39.1 less than a year	
39.2 1-5 years	
39.3 more than 5 years	

40. Of what material is your pillow?

TICK ONE BOX ONLY

40.1 wool
40.2 feather
40.3 fibre
40.4 Cotton
40.5 straw
40.6 other (specify)

41. The walls of your bedroom:

	NO	YES
41.1 are painted?		
41.2 have wall paper?		
41.3 are varnished?		
41.4 none of the above		

	NO	YES
42. Is your house humid?		

	NO	YES	DON'T KNOW
43. Has there ever been any mould or mildew on any surface, other than food, inside the home?			

IF 'NO' OR 'DON'T KNOW' GO TO QUESTION 45, IF 'YES':

43.1 Which rooms have been affected?

	NO	YES
43.1.1 bathroom(s)		
43.1.2 bedroom(s)		
43.1.3 living area(s)		
43.1.4 kitchen		
43.1.5 basement or attic		
43.1.6 other: _____		

	NO	YES
44. Has there been mould inside the house in the last 12 months?		

	NO	YES
45. Does your house get wet inside if it rains?		

	NO	YES	DON'T KNOW
46. Has there ever been any water damage to the building or its contents, for example, from broken pipes, leaks or floods?			

Animals

	NO	YES
47. Do you keep a cat?		

IF 'NO' GO TO QUESTION 48, IF 'YES':

	NO	YES
47.1 Is your cat ever allowed into your bedroom?		
47.2 Do all your cats stay outside the house?		

	NO	YES
48. Do you keep a dog?		

IF 'NO' GO TO QUESTION 49, IF 'YES':

	NO	YES
48.1 Is your dog ever allowed into your bedroom?		
48.2 Do all your dogs stay outside the house?		

	NO	YES
49. Were there any cats in your house		
49.1 during your first year of life?		
49.2 when you were between 1 and 4 years old?		
49.3 when you were between 5 and 15 years old?		

	NO	YES
50. Were there any dogs in your house		
50.1 during your first year of life?		
50.2 when you were between 1 and 4 years old?		
50.3 when you were between 5 and 15 years old?		

51.1 Before you were 10 years old, did anyone in your household grow any of the following animals?

	NO	YES
51.1 pigs		
51.2 cows		
51.3 others (specify)		
51.4 did not grow any animals		

52. When you are near animals, such as cats, dogs or horses, near feathers, including pillows, quilts or duvets, or in a dusty part of the house, do you *ever*

	NO	YES
52.1 start to cough?		
52.2 start to wheeze?		
52.3 get a feeling of tightness in your chest?		
52.4 start to feel short of breath?		
52.5 get a runny or stuffy nose or start to sneeze?		
52.6 get itchy or watering eyes?		
52.7 get skin allergy?		

53. When you are near dust in the house or after scrubbing?

	NO	YES
53.1 start to cough?		

53.2 start to wheeze?
53.3 get a feeling of tightness in your chest?
53.4 start to feel short of breath?
53.5 get a runny or stuffy nose or start to sneeze?
53.6 get itchy or watering eyes?
53.7 get skin allergy?

54. When you are near trees, grass or flowers, or when there is a lot of pollen about, do you ***ever***

NO YES

a) start to cough?
b) start to wheeze?
c) get a feeling of tightness in your chest?
d) start to feel short of breath?
e) get a runny or stuffy nose or start to sneeze?
f) get itchy or watering eyes?
g) get skin allergy?

IF 'YES' TO ANY OF THE ABOVE:

54.1 Which time of year does this happen?

NO YES

a) winter
b) spring
c) summer
d) autumn

<u>Diet</u>

55. How often do you eat pre-packaged food, such as tinned food or pre-prepared frozen meals?

TICK ONE BOX ONLY

a) every day or most days
b) at least once a week
c) occasionally
c) never

56. Have you ever had an illness or trouble caused by eating a ***particular*** food or foods? NO YES

IF 'NO' GO TO QUESTION 57, IF 'YES':

56.1 Have you nearly always had the same illness or trouble after eating this type of food? NO YES

56.2 What type of food was this?

_____

56.3 Did this illness or trouble include

	NO	YES
a) a rash or itchy skin?	☐	☐
b) diarrhoea or vomiting?	☐	☐
c) runny or stuffy nose?	☐	☐
d) severe headaches?	☐	☐
e) breathlessness?	☐	☐
f) other: _____	☐	☐

Smoking

57. Have you ever smoked for as long as a year?

NEVER ☐ NO ☐ YES ☐

['YES' means at least 20 packs of cigarettes or 12 oz (360 grams) of tobacco in a lifetime, or at least one cigarette per day or one cigar a week for one year]

IF 'NEVER' GO TO QUESTION 58, IF 'NO' GO TO QUESTION 57.4, IF 'YES':

57.1 How old were you when you started smoking? YEARS ☐☐

57.2 Do you **now** smoke, as of ***one month ago***? NO ☐ YES ☐

57.3 How much do you ***now*** smoke on average

a) number of cigarettes per day NUMBER ☐☐
b) number of cigarillos per day ☐☐

c) grams of pipe tobacco a week GRAMS ☐☐☐

57.4 How old were you when you stopped or cut down smoking? YEARS ☐☐

57.5 ***On average*** of the entire time you smoked, before you stopped or cut down, how much did you smoke?

a) number of cigarettes per day NUMBER ☐☐
b) number of cigars a week ☐☐
c) grams of pipe tobacco a week ☐☐

57.6 Do you or did you inhale the smoke? NO ☐ YES ☐

58. Have you been **regularly** exposed to tobacco smoke in the last ***12 months***? ['Regularly' means on most days or nights]

NO YES ☐ ☐

IF 'NO' GO TO QUESTION 59, IF 'YES':

58.1 Not counting yourself, how many people in your household smoke regularly?

NUMBER ☐☐

58.2 Do people smoke regularly in the room where you work?

NO YES ☐ ☐

58.3 How many hours per day are you exposed to ***other people's*** tobacco smoke?

HOURS ☐☐

59. Medicines

If you have received any medicine for the alleviation of your breathing problems, congestion or allergy in the last 12 months, please indicate them:

Name of the medicine	**How do you take them?** Inhaled	Nebulised	Syrup	Tablets	Times/day	Last month you Took them

60. Do you take aspirin?

60. 1 How often? Daily, weekly, occasionally

61. Do you take paracetamol?

61. 1 How often? Daily, weekly, occasionally

62. When were you born?

YEAR ☐☐☐☐

63. Please indicate, are you

FEMALE MALE ☐☐

64. How old was your mother when you were born?

YEARS

65. How many times did you move to another house in the first 5 years of your life?

TICK ONE BOX ONLY

a) never
b) once
c) more than once
c) don't know

DON'T
NO YES KNOW

66. Did you normally share your bedroom with older children when you were younger than 5 years old?

DON'T
NO YES KNOW

67. Did you go to a nursery with other children before you were 5 years old?

DON'T
NO YES KNOW

68. Did you ever have any important respiratory infection before 5 years old?

DON'T
NO YES KNOW

69. Were you ever admitted into hospital due to a pulmonary disease before you were 2 years old?

NO YES

70. Are you a full-time student?

71. What is your current work?. If you don't work at present, what was your last job?

NO YES

71.1 The work you currently have is:

1) Professional
2) Professional in the public service
3) Business
4) Employee at a middle level in the private or public sector
5) Employee of basic level
6) Specialised hand worker
7) Non specialised hand worker
8) House wife
9) "Temporary worker"
10) Unemployed

72. Have you ever had any of the following jobs?:

NO YES

1) Worker in a farmer ☐ ☐
2) Painter ☐ ☐
3) Working with metallic objects ☐ ☐
4) Working with plastic objects ☐ ☐
5) Cleaner, domestic work ☐ ☐
6) Bird grow farms ☐ ☐
7) Administrative job ☐ ☐
8) Baker ☐ ☐

73.Has any of the jobs you have worked on caused you wheezing or chest tightness? NO ☐ YES ☐

73.1 Which job?

.._____

74. Have you needed to leave your job because it was causing you breathing problems? NO ☐ YES ☐

IF 'NO', GO TO QUESTION 75, IF 'YES':

74.1 Which job was causing you this problem?

_____

75. Have you ever been exposed to vapours, gases, dust or fume? NO ☐ YES ☐

IF 'NO', GO TO QUESTION 76, IF 'YES':

75. Which job was this? _____

Physical activity

TICK ONE BOX ONLY

76. In relation to your work, would you consider it to bes:

1) very active ☐
2) relatively active ☐
3) not very active ☐
4) not active at all ☐

77. Do you practice any sport or physical activity outside your work? NO ☐ YES ☐

TICK ONE BOX ONLY

78. How often do you exercise until you break sweat or are left breathlessness?

a) every day or most days .
b) 4-6 times a week
c) 2-3 times a week
d) once a week
e) once a month
f) less than once a month
g) never

If you answered less than once a month or never :

NO YES

79. Do you avoid exercising because you have difficulty with your breathing or because it gives you asthma?

80. During the week, how many hours do you normally watch TV per day?

81. Do you watch TV during the day on weekends?

82. Do you have a computer at home?

83. If you answered yes, how many hours of your free time do you dedicate to use the PC?

84. What does describe best the place where you used to live when were less than 5 years old?

a) Countryside
b) Village in the country (with more than 50 and less than 100 people)
c) Nearby a big city
d) Centre of a big city

85. How many people live in your house?

a) In the same house
b) Sharing the same food

86. Would you consider your family as?

b) Nuclear (parents, children)
c) Extended family (parents, children, other relatives)
d) extended (parents, children, other persons that are not relatives)

87. How many brothers and sisters do you have?

NUMBER

88. How many elder brothers do you have?

NUMBER

89. How many elder sisters do you have?

NUMBER ☐☐

90. What was your last course approved?

YEAR ☐☐

91. Are you the breadwinner in your family?

NO ☐ YES ☐

92. If is not yourself, what activity does the breadwinner?

.....

93. Indicate within which range the income of your family is:

TICK ONE BOX ONLY

a) Less than $100,000
b) Between $100,000 y $200,000
c) Between $200,000 and $500,000
d) Between $500,000 and $800,000
e) Between $800,000 and $1,300,000
f) Between $1,300,000 and $2,000,000
g) Above $2,000,000

☐
☐
☐
☐
☐
☐
☐

TICK ONE BOX ONLY

94. What was the last course that your father approved in school?

☐ YEARS

95. How old is your mother?

☐ YEAR

96. What was the last course that your mother approved in school?

☐

Your house:

97. Is your house a

TICK ONE BOX ONLY

a) House or flat with 4 bedrooms?
b) Solid flat or house (2 to 3 bedrooms)?
c) Council flat or house?
d) Light wooden house?
e) Very light material and precarious house?

☐
☐
☐
☐
☐

98. In the house where you live now, you are:

TICK ONE BOX ONLY

a) Owner, paying mortgage
b) Letting
c) Owner with no mortgage
d) Friend or relative receiving free board and lodging
e) Have taken that piece of land illegally

☐
☐
☐
☐
☐

99. How many bedrooms does your house have? (excluding bathroom and kitchen) ☐

100. Which of the following consumables are in your house?

	NO	YES
a) Fridge	☐	
b) Hot water heating device in working conditions	☐	
c) Washing mashing in working order	☐	
d) Microwave	☐	

101. Does your family have any cars? ☐ ☐

IF 'NO', GO TO QUESTION 102, IF 'YES':

101.1 How many vehicles do you have? ☐

101.2 How many of these vehicles are for a working purpose? ☐

	NO	YES
102. Is your family registered with the Social Security Agency?	☐	☐

	NO	YES
103. Over the last five years, have you gained more than 8 kgs?	☐	☐

104. How much did you weight 5 years ago? ☐☐☐,☐

Finally, some measurements:

105. Arterial pressure

Systolic pressure (mmHg) 1st measurement ☐☐☐

Diastolic pressure (mmHg) 1st measurement ☐☐☐

Systolic pressure (mmHg) 2nd measurement ☐☐☐

Diastolic pressure (mmHg) 2nd measurement ☐☐☐

Systolic pressure (mmHg) 3rd measurement ☐☐☐

Diastolic pressure (mmHg) 3rd measurement ☐☐☐

106. Brachial perimeter (cms) ☐☐☐.☐

107. Tricipital fold (mm) ☐☐☐.☐

108. Waist circumference (cm) ☐☐☐.☐

Name of Fieldworker

.....

Observations .....

.....

Questionnaire checked and approved by supervisor .....

B. LUNG FUNCTION PROTOCOL (PART 1)

Identification Number ☐☐☐☐

Date ☐☐☐☐☐☐

Day Month Year

Before starting this questionnaire please ask the following questions:

NO YES

Have you had a cigarette in the last hour? ☐☐

NO YES

Have you used an inhaler (puffer) in the last 24 hours ☐☐

If yes, delay lung function tests until one hour after the last cigarette or inhaler use (responses do not have to be included in data recorder).

1. How many times have you been woken at night with shortness of breath in the last two weeks? ☐☐

2. During the last two weeks, has your breathing been TICK ONE BOX ONLY

1. worse than usual? ☐
2. same as usual? ☐
3. better than usual? ☐

NO YES

3. Have you had wheezing or whistling in your chest in the last 3 days? ☐☐

4. Have you woken up with a feeling of tightness in your chest in the last 3 days?

NO YES

☐☐

5. Have you been woken by an attack of shortness of breath in the last 3 days NO YES ☐☐

6. Have you woken by an attack of coughing in the last 3 days? NO YES ☐☐

7. Have you had an attack of asthma in the last 3 days ? NO YES ☐☐

8. Have you taken any medicine (including inhalers, aerosols or tablets) for asthma in the last 3 months? NO YES ☐☐

9. Have you had any symptoms of hay fever or nasal allergy in the last 3 days? NO YES ☐☐

10. Have you had a respiratory infection in the last 3 weeks? NO YES ☐☐

11. Have you used an inhaler in the last 24 hours ? NO YES ☐☐

If 'NO', go to question 12, if 'YES':

11.1 What inhaler(s) did you use and how may hours did you use it?

	Drug	Hours
.....	.. ☐☐	☐☐
.....	..... ☐☐	☐☐

If the subject has used a beta-2-agonist inhaler or an anti-muscarinic inhaler in the last six hours,do:

a) Wait until four hours since last use has elapsed
b) Reschedule for another day if the subject is willing.

12. Have you used any other medicine (including pills, capsules or suppositories) to help your breathing, or any oral anti-muscarinic in the last 24 hours?

NO YES
☐☐

If 'NO', go to question 13, if 'YES',

12.1 What medicine(s) did you take and how many hours ago did you take it?

Drug Hours

.... ☐☐ ☐☐

.... ☐☐ ☐☐

If the subject has taken an oral beta-2-agonist, an oral theophylline or an oral anti-muscarinic, reschedule for another day.

NO YES

13. Have you had an heart attack in the last three months? ☐☐

NO YES

14. Are you currently taking any medicine(s) for your heart? ☐☐

NO YES

15. Are you currently taking any medicines for epilepsy? ☐☐

16. Do you usually take any medicines containing beta-blockers, including eye-drops?

NO YES

☐☐

Only for women.

NO YES

17. Are you pregnant? ☐☐

NO YES

18. Are you breast feeding? ☐☐

If the answer is 'YES' to questions 13-18, measure baseline spirometry only, **do not challenge with methacholine.**

B. LUNG FUNCTION PROTOCOL, DATA COLLECTION SHEETS (PART 2)

Identification Number □□□□

Date: □□□□□□
Day Month Year

Date of birth: □□□□□□
Day Month Year

1.- Height (m) □□□,□
2.- Weight (kg) □□□,□
3.- Age (years) □□
4.- Sex Male= 1 Female = 2 □

5. Time of the day
Hrs. Min □□□□

6.- PREDICTED FEV_1
□.□□

Each participant now has five attempts to produce a technically satisfactory manoeuvre. If after five attempts there have not produced at least two manoeuvres – four more attempts are allowed. Maximum number of attempts allowed is nine

7.- INITIAL FEV_1 AND FVC

	FEV_1 (L)	**FVC (L)**	**PEF (L)**
1	□.□□	□.□□	□□□
2	□.□□	□.□□	□□□
3	□.□□	□.□□	□□□
4	□.□□	□.□□	□□□
5	□.□□	□.□□	□□□

PEF = Peak expiratory flow (Vmax)

7.1 Number of rejected attempts
□

8.- Best initialFEV_1 INICIAL as% of predicted FEV_1
□□□

If best initial FEV_1:

a) less than 70% predicted or
b) less than 1,5 litres

GO TO BRONCHODILATOR CHALLENGE – DO NOT DO METHACHOLINE CHALLENGE

<u>METHACHOLINE CHALLENGE</u>

Give four inhalations of diluent. Two minutes later record FEV_1

9.- Control FEF_1 following inhalation of diluent

9.1. Record two technically satisfactory manoeuvres

1. □.□□
2. □.□□

9.2. Number of rejected attempts □

10.BEST CONTROL (post-diluent)FEV_1 as % of inicial FEV_1 □□□

If best control FEV_1 <90% of bet initial FEV_1 stop methacholine challenge and go to reversal of bronchoconstriction

11.-Methacholine batch number □□□

Date of preparation of original solution □□□□□□

Day Month Year

Concentration of methacholine	Absolute value of FEV_1	% of change compared to basal value	Rejected attempts
0.5	□.□□	□□□	□
1	□.□□	□□□	□
4	□.□□	□□□	□
8	□.□□	□□□	□
16	□.□□	□□□	□

12.- Why was methacholine challenge stopped?: TICK ONE BOX ONLY

a) end of test reached (inhaled 16 mg) 1 □

b) >20% fall in FEV_1 occurred 2 □

c) two satisfactory manoeuvres not achieved 3 □

d) participant asked to stop 4 □

e) .other..... 5 ☐

IF PARTICIPANT'S FEV_1 HAS FALLEN BY MORE THAN 10%

Reversal of bronchoconstriction

GIVE 400 µg SALBUTAMOL VIA INHALER AND 10 MINUTES LATER RECORD FEV_1

13.- FEV_1 AND FVC

13.1. Record first two tecnically satisfactory manoeuvres (up to five attempts)
FEV_1 ☐,☐☐ FVC ☐, ☐☐

FEV_1 ☐,☐☐ FVC ☐,☐☐

13.2. Number of rejected attempts ☐

14.- Best post-bronchodilator FEV_1 as % of initial FEV_1 ☐☐☐

15.- Has the participant's FEV_1 returned to within 10% of initial FEV_1 NO ☐ YES ☐

IF 'YES' THE PARTICINAT MAY LEAVE THE CENTRE.

IF 'NO' FURTHERACTION MUST BE TAKEN TORESTORE BASELINE LUNG FUNCTION

BRONCHODILATOR CHALLENGE ONLY

GIVE 400 µg DE SALBUTAMOL VIA INHALER VOLUMATIC AND 10 MINUTES LATER RECORD FEV_1

16.- FEV_1 and FVC

16.1. Record first two technically satisfactory manoeuvres (up to five attempts)
FEV_1 ☐,☐☐ FVC ☐,☐☐

VEF_1 ☐,☐☐ FVC ☐,☐☐
16.2. Number of rejected attempts ☐

Fieldworker:.....

Signature:.....

C: FOOD FREQUENCY QUESTIONNAIRE

Code number_____

Name _____ Date_____

Food item	Frequency of consumption	Amount eaten each time Home size	Grams	Total grams consumed according to frequency	Daily grams	Observations
Fruits						
Orange	3 per week	2 units	150g each	900	128.6	
Lemon						
Kiwi						
Apple						
Strawberries						
Mandarin						
Vegetables						
Beetroot						
Chard						
Sweet pepper (green/red)						
Garlic						
Onion						
Tomato						
Potato Stew Fried Mashed						
Pumpkin						
Carrot						
Avocado						

Food item	Frequency of consumption	Amount eaten each time Home size	Grams	Total grams consumed according to Frequency	Daily grams	Observations
Legumes						
Beans (Kidney)						
Lentils						
Chick-peas						
Meats/Fish/Eggs						
Chicken Whole (meat and Skin) Only meat						
Beef Steak Stew Minced						
Pork Rib Lean and fat						
Salmon						
Other (Hake)						
Clam						
Egg						
Cereals						
Bread White Kneaded yeast bread Brown						
Spaguetti						
Rice						
Cake/pastries						

Food item	Frequency of consumption	Amount eaten each time Home size	Grams	Total grams consumed according to frequency	Daily grams	Observations
Fatty products						
Oil for cooking Olive Sunflower **Oil for dressing** Olive Sunflower						
Reheated oil for cooking						
Bacon						
Longaniza*						
Frankfurter						
Ham Pork/Turkey/Chicken						
Offal (average)						
Margarine 70-80% fat blended 35-40% fat blended Butter (regular)						
Mayonnaise Normal Light						
Dairy products						
Cheese Gouda						

Cottage (reduced fat)						
Food item	**Frequency of consumption**	**Amount eaten each time** Home size	Grams	**Total grams consumed according to Frequency**	**Daily grams**	**Observations**
Yogurt Whole milk Low fat						
Sweets						
Sugar						
Jam						
Honey						
Soft drink (sparkling beverages)						
Juice (artificial)						
Others						
Tea (strong/light)						
Coffee						
Red wine						
Salt						

* Longaniza is equivalent to Chorizo

Do you take any nutritional supplements? YES NO If yes, name it and specify the amount and frequency of consumption (per day)_____

For the interviewer

Please state below any additional comments you may consider useful in relation to the dietary intake of the participant.

APPENDIX 2

Association between BHR and respiratory symptoms with food and nutrient intake, flavonoids and plasma biomarkers

This appendix provides analyses on individual food items and nutrients in relation to the outcomes that are studied in the primary hypothesis of this thesis. As no food item studied showed evidence a for non-linear relationship with any of the outcomes in analyses using quintiles of intake, most of them were analysed as the continuous increase for 100g of their consumption. Garlic and salt were analysed as per 10g of increase, because their normal consumption is very unlikely to reach 100 g a day in the usual dietary pattern of adults. There were some food items whose intake were insufficient to classify them into quintiles, so were dichotomised as eating any amount equal to or above 1g/day, or eating less than 1 g/day of that food item.

The macronutrients studied were proteins, carbohydrates, lipids (MUFA, SFA, PUFA, omega 3, omega 6 fatty acids, ratio n6/n3). Micronutrients analysed included vitamins and minerals. Among the first were retinol, beta carotene, and total vitamin A, vitamins B (B1, B2, niacin, B6, B12), Vitamin C, vitamin E (alpha- tocopherol), folic acid, and pantotenic acid. The minerals analysed were calcium, copper, iron, magnesium, phosphorus, potassium, selenium, sodium, and zinc.

Model 1 shows crude associations. Two models of multiple linear regression analyses were used to assess the association between measurement of BHR, with individual foods, as well as with antioxidant nutrients. Model 2 adjusted for age, sex, socio-economic confounders, weight at birth, BMI, FEV_1%, FEV_1%FVC and atopy. Model 3 added TEI. This was aimed to assess whether adjustment for TEI would affect the associations found, if any. Bonferroni correction was applied in those tests in Appendices 2 and 3 that showed nominal statistical significance.

Eeach respiratory outcome was analysed using three statistical models assessing associations with individual food items, nutrients and grouped flavonoids. The analyses of separate multivariable models were intended to evaluate the possibly

confounding effect that different sets of variables could have on both respiratory symptoms and dietary intake. The statistical models included the same variables than that for BHR slope except FEV_1%, FEV_1%FVC and atopy.

To examine the association between the outcomes studied and biomarkers as independent variables, only two models were analysed. The rationale for this is that TEI is unlikely to affect levels of biomarkers of oxidative stress or antioxidant status such as those included in the current study.

A few associations of nominal statistical significance were found, but all the P-values were raised above the level of significance after Bonferroni correction. Intakes of garlic (Table A (1)), and vitamin C (Table A (2)) were negatively associated with BHR slope. This association remained statistically significant across the three models studied, but disappeared after Bonferroni correction. Contrary, positive associations of nominal statistical significance were found between BHR slope and selenium and sodium intake (Table A (2)), but the P-value was raised above 0.3 after Bonferroni correction.

Waking with shortness of breath was negatively associated with intake of rice and positively associated with sugar (Table C (1)) (Model 3: OR 0.37, 95% CI 0.17 to 0.81, and OR 2.26, 95% CI 1.18 to 4.35, respectively). Having at least one respiratory symptom was negatively associated with intake of lentils (OR 0.42, 95% CI 0.20 to 0.88) and positively and statistically significantly associated with intake of sweet pepper, sugar, tea and coffee (Table D (1)). In addition, a negative association was found between this outcome and intake of calcium (OR 0.78, 95% CI 0.64 to 0.95), which remained statistically significant across the three models (Table D (2)). Finally, plasma levels of F2-ip were negatively associated with having at least one respiratory symptom both univariately and in the multivariable model (Table D (4)). Again, all these associations were no longer of statistical significance after Bonferroni correction.

A. Association between BHR as slope and independent variables

Table A (1): Association between BHR (mg^{-1}) and food items

Food group	Food Item	Model 1			Model 2			Model 3		
	Per 100g except^#	b-coeff	95% CI	p value	b-coeff	95% CI	p value	b-coeff	95% CI	p value
Fruits	Orange	-0.19	-0.06 to 0.02	0.34	-0.02	-0.06 to 0.02	0.35	-0.02	-0.06 to 0.02	0.36
	Lemon	-0.07	-0.35 to 0.21	0.62	-0.07	-0.36 to 0.21	0.63	-0.07	-0.36 to 0.22	0.63
	Kiwi^	-0.01	-0.19 to 0.18	0.96	-0.03	-0.22 to 0.16	0.77	-0.03	-0.22 to 0.16	0.77
	Apple	0.02	-0.05 to 0.08	0.60	0.01	-0.06 to 0.08	0.75	0.01	-0.06 to 0.08	0.73
	Strawberries^	-0.16	-0.35 to 0.02	0.08	-0.17	-0.35 to 0.02	0.08	-0.17	-0.35 to 0.02	0.08
	Mandarin	-0.02	-0.07 to 0.04	0.59	-0.02	-0.08 to 0.04	0.47	-0.02	-0.08 to 0.04	0.48
Vegetables	Beetroot^	-0.04	-0.23 to 0.15	0.67	-0.06	-0.25 to 0.13	0.55	-0.06	-0.25 to 0.13	0.55
	Chard	-0.02	-0.31 to 0.27	0.90	-0.02	-0.32 to 0.27	0.88	-0.02	-0.32 to 0.27	0.88
	S. pepper	-0.03	-0.45 to 0.39	0.89	-0.07	-0.50 to 0.35	0.73	-0.07	-0.50 to 0.35	0.74
	Garlic^#	**-0.16**	**-0.29 to -0.03**	**0.02**	**-0.18**	**-0.31 to –0.04**	**0.01**	**-0.18**	**-0.32 to -0.04**	**0.01***
	Onion	0.12	-0.02 to 0.25	0.09	0.11	-0.03 to 0.25	0.12	0.12	-0.02 to 0.26	0.11
	Tomato	-0.02	-0.05 to 0.01	0.26	-0.02	-0.06 to 0.01	0.18	-0.03	-0.06 to 0.01	0.17
	Potato	-0.03	-0.10 to 0.04	0.38	-0.04	-0.11 to 0.03	0.28	-0.04	-0.12 to 0.03	0.26
	Pumpkin	-0.35	-0.80 to 0.11	0.14	-0.42	-0.89 to 0.04	0.08	-0.45	-0.93 to 0.04	0.07
	Carrot	0.03	-0.15 to 0.20	0.78	0.01	-0.16 to 0.19	0.87	0.02	-0.16 to 0.20	0.85
	Avocado	-0.01	-0.06 to 0.04	0.67	-0.02	-0.07 to 0.04	0.52	-0.02	-0.08 to 0.04	0.51
Legumes	Bean	-0.03	-0.44 to 0.39	0.91	-0.08	-0.52 to 0.37	0.73	-0.08	-0.53 to 0.38	0.74
	Lentil	0.02	-0.51 to 0.55	0.94	-0.11	-0.66 to 0.43	0.69	-0.11	-0.66 to 0.44	0.69
	Chickpeas^	-0.01	-0.22 to 0.19	0.89	-0.02	-0.23 to 0.18	0.82	-0.02	-0.23 to 0.19	0.83
Meats	Red meat	0.01	-0.18 to 0.21	0.90	0.03	-0.17 to 0.24	0.77	0.04	-0.18 to 0.25	0.74
	Chicken	0.45	-0.05 to 0.94	0.08	0.44	-0.06 to 0.95	0.08	0.45	-0.06 to 0.95	0.08
	Pork ribs	-0.02	-0.51 to 0.47	0.94	-0.01	-0.51 to 0.49	0.97	-0.003	-0.52 to 0.52	0.99
	Fish	-0.12	-0.43 to 0.20	0.47	-0.10	-0.43 to 0.22	0.53	-0.10	-0.43 to 0.22	0.53
	Eggs	0.13	-0.12 to 0.37	0.31	0.12	-0.14 to 0.38	0.35	0.13	-0.13 to 0.40	0.32

* Bonferroni corrected P-value= 0.47

Continuation Table A (1)

Food group	Food Item	Model 1			Model 2			Model 3		
	Per 100g except^	b-coeff	95% CI	p value	b-coeff	95% CI	p value	b-coeff	95% CI	p value
Cereals	Bread	0.01	-0.03 to 0.05	0.66	0.01	-0.03 to 0.06	0.63	0.03	-0.04 to 0.09	0.42
	Pasta^	0.45	-0.11 to 1.01	0.12	0.43	-0.14 to 1.00	0.14	0.44	-0.13 to 1.01	0.13
	Rice^	0.14	-0.39 to 0.68	0.60	0.08	-0.46 to 0.63	0.76	0.09	-0.46 to 0.63	0.76
	Cake^	-0.15	-0.33 to 0.04	0.12	-0.15	-0.34 to 0.04	0.11	-0.15	-0.34 to 0.04	0.11
Fatty foods	Oil	-0.52	-1.36 to 0.31	0.22	-0.57	-1.42 to 0.28	0.19	-0.60	-1.49 to 0.28	0.18
	Bacon^	-0.15	-0.46 to 0.17	0.37	-0.16	-0.49 to 0.16	0.33	-0.16	-0.49 to 0.17	0.33
	Sausage^	-0.05	-0.23 to 0.14	0.63	-0.06	-0.25 to 0.13	0.54	-0.06	-0.25 to 0.13	0.54
	Frankfurter	0.06	-0.51 to 0.64	0.83	0.06	-0.53 to 0.65	0.84	0.001	-0.01 to 0.01	0.80
	Ham	0.02	-0.30 to 0.35	0.88	0.02	-0.31 to 0.35	0.90	0.03	-0.33 to 0.39	0.87
	Offal	-0.02	-0.24 to 0.19	0.85	-0.06	-0.27 to 0.16	0.61	-0.06	-0.27 to 0.16	0.62
	Margarine	0.18	-0.38 to 0.73	0.54	0.15	-0.40 to 0.71	0.59	0.17	-0.40 to 0.73	0.57
	Mayonnaise	-0.12	-0.96 to 0.72	0.78	-0.23	-1.08 to 0.63	0.60	-0.23	-1.13 to 0.66	0.61
Dairy foods	Milk^	-0.06	-0.25 to 0.13	0.52	-0.09	-0.28 to 0.10	0.36	-0.09	-0.28 to 0.10	0.36
	Cheese	0.09	-0.28 to 0.46	0.64	0.08	-0.29 to 0.46	0.66	0.10	-0.29 to 0.49	0.62
Sweets	Sugar	0.12	-0.27 to 0.52	0.54	0.10	-0.30 to 0.50	0.62	0.11	-0.30 to 0.52	0.60
	Jam^	-0.09	-0.28 to 0.09	0.32	-0.12	-0.31 to 0.07	0.21	-0.12	-0.31 to 0.07	0.21
	Honey^	-0.05	-0.28 to 0.19	0.70	-0.08	-0.32 to 0.16	0.53	-0.08	-0.32 to 0.17	0.53
	Coke	-0.01	-0.03 to 0.01	0.51	-0.01	-0.03 to 0.01	0.33	-0.01	-0.03 to 0.01	0.33
	Juices^	0.13	-0.06 to 0.32	0.17	0.15	-0.04 to 0.35	0.13	0.16	-0.04 to 0.35	0.12
Beverages	Tea^	-0.04	-0.23 to 0.16	0.72	-0.02	-0.22 to 0.17	0.81	-0.02	-0.22 to 0.17	0.82
	Coffee^	-0.09	-0.27 to 0.09	0.33	-0.11	-0.30 to 0.08	0.26	-0.11	-0.30 to 0.08	0.26
	Red wine^	-0.04	-0.23 to 0.14	0.65	-0.08	-0.29 to 0.13	0.45	-0.08	-0.29 to 0.13	0.45
Other	Salt#	0.09	-0.06 to 0.24	0.24	0.15	-0.09 to 0.39	0.21	0.15	-0.08 to 0.39	0.20

^ Represents a dichotomised variable (eats/doesn't eat that food item once a day)
# Garlic and salt were analysed as per 10g increase

Table A (2): Association between BHR (mg^{-1}) and per doubling increase in nutrient intake

Nutrient group	Nutrient	Model 1			Model 2			Model 3		
		b-coeff	95% CI	p value	b-coeff	95% CI	p value	b-coeff	95% CI	p value
Energy and macro-nutrients	Energy	0.04	-0.10 to 0.18	0.59	0.03	-0.14 to 0.20	0.72	0.27	-0.17 to 0.71	0.23
	Proteins	0.09	-0.06 to 0.23	0.26	0.09	-0.07 to 0.25	0.28	0.20	-0.03 to 0.44	0.09
	Carbohydrates	0.05	-0.09 to 0.20	0.47	0.05	-0.12 to 0.21	0.59	0.20	-0.12 to 0.51	0.22
	Total lipids	-0.01	-0.12 to 0.10	0.89	-0.02	-0.14 to 0.10	0.75	-0.04	-0.25 to 0.16	0.69
	PUFA	-0.01	-0.12 to 0.11	0.90	-0.02	-0.14 to 0.10	0.80	-0.02	-0.18 to 0.14	0.80
	MUFA	-0.01	-0.11 to 0.09	0.89	-0.02	-0.12 to 0.09	0.75	-0.03	-0.21 to 0.14	0.70
	SFA	0.004	-0.09 to 0.10	0.94	-0.01	-0.11 to 0.10	0.92	-0.002	-0.17 to 0.17	0.98
	Omega 6	-0.03	-0.11 to 0.06	0.55	-0.03	-0.12 to 0.06	0.51	-0.03	-0.13 to 0.06	0.51
	Omega 3	0.02	-0.03 to 0.08	0.39	0.02	-0.04 to 0.07	0.50	0.02	-0.04 to 0.08	0.46
	Ratio n6/n3	-0.03	-0.08 to 0.02	0.25	-0.03	-0.08 to 0.02	0.32	-0.03	-0.08 to 0.02	0.32
	Cholesterol	0.06	-0.04 to 0.16	0.22	0.06	-0.05 to 0.17	0.29	0.09	-0.04 to 0.23	0.18
Vitamins	β–Carotene	0.01	-0.07 to 0.09	0.83	0.004	-0.07 to 0.08	0.92	0.01	-0.08 to 0.09	0.89
	Retinol	0.04	-0.03 to 0.10	0.27	0.03	-0.04 to 0.10	0.42	0.04	-0.04 to 0.11	0.35
	Total vitamin A	0.03	-0.06 to 0.12	0.57	0.02	-0.07 to 0.11	0.70	0.02	-0.08 to 0.12	0.65
	Vitamin B1	0.05	-0.08 to 0.18	0.49	0.05	-0.10 to 0.20	0.55	0.18	-0.10 to 0.46	0.20
	Vitamin B2	0.07	-0.06 to 0.20	0.32	0.06	-0.09 to 0.21	0.42	0.22	-0.05 to 0.49	0.10
	Niacin	0.06	-0.08 to 0.21	0.37	0.07	-0.09 to 0.23	0.40	0.30	-0.02 to 0.62	0.07
	Vitamin B6	-0.03	-0.18 to 0.11	0.63	-0.07	-0.22 to 0.08	0.37	-0.13	-0.34 to 0.09	0.25
	Vitamin B12	-0.02	-0.09 to 0.05	0.57	-0.03	-0.10 to 0.05	0.48	-0.03	-0.11 to 0.05	0.48
	Vitamin C	-0.08	-0.16 to 0.003	0.06	**-0.11**	**-0.19 to -0.02**	**0.01**	**-0.12**	**-0.22 to -0.03**	**0.01***
	Vitamin E	-0.03	-0.16 to 0.09	0.63	-0.05	-0.18 to 0.08	0.43	-0.08	-0.26 to 0.09	0.35
	Folic Acid	0.06	-0.06 to 0.18	0.32	0.07	-0.07 to 0.20	0.35	0.17	-0.04 to 0.39	0.12
	Pantotenic Acid	-0.004	-0.14 to 0.13	0.95	-0.04	-0.19 to 0.11	0.64	-0.07	-0.30 to 0.16	0.55
Minerals	Calcium	0.03	-0.09 to 0.15	0.61	0.02	-0.11 to 0.14	0.80	0.03	-0.12 to 0.18	0.70
	Copper	0.002	-0.14 to 0.14	0.98	-0.03	-0.19 to 0.13	0.72	-0.05	-0.30 to 0.20	0.67
	Iron	0.05	-0.10 to 0.19	0.50	0.04	-0.12 to 0.21	0.61	0.19	-0.13 to 0.50	0.25
	Magnesium	-0.02	-0.16 to 0.12	0.79	-0.06	-0.21 to 0.10	0.47	-0.11	-0.35 to 0.12	0.34
	Phosphorus	0.06	-0.09 to 0.21	0.43	0.06	-0.11 to 0.22	0.51	0.16	-0.10 to 0.42	0.24
	Potassium	-0.05	-0.19 to 0.08	0.43	-0.09	-0.24 to 0.05	0.19	-0.17	-0.36 to 0.03	0.09
	Selenium	0.11	-0.03 to 0.24	0.12	0.14	-0.02 to 0.29	0.08	**0.34**	**0.11 to 0.58**	**0.01***
	Sodium	**0.18**	**0.03 to 0.32**	**0.02**	**0.18**	**0.02 to 0.34**	**0.03**	**0.25**	**0.07 to 0.44**	**0.01***
	Zinc	0.05	-0.09 to 0.19	0.51	0.05	-0.11 to 0.21	0.57	0.11	-0.12 to 0.35	0.35

* Bonferroni-corrected P-value=0.32

Table A (3): Association between BHR (mg^{-1}) and per-quintile increase in flavonoid intake

Flavonoids	Model 1			Model 2			Model 3		
	b-coeff	95% CI	p value (Trend)	b-coeff	95% CI	p value (Trend)	b-coeff	95% CI	p value (Trend)
Total catechins	-0.02	-0.08 to 0.05	0.57	-0.03	-0.09 to 0.04	0.44	-0.03	-0.10 to 0.04	0.44
Flavonols	0.001	-0.06 to 0.07	0.97	0.001	-0.07 to 0.07	0.97	0.003	-0.07 to 0.07	0.94
Flavones	0.04	-0.03 to 0.10	0.29	0.03	-0.04 to 0.09	0.41	0.03	-0.04 to 0.10	0.39

Table A (4): Association between BHR (mg^{-1}) and per-quintile increase of biomarkers

Biomarkers	Model 1			Model 2		
	b-coeff	95% CI	p value (Trend)	b-coeff	95% CI	p value (Trend)
FRAP	-0.02	-0.12 to 0.08	0.70	-0.01	-0.12 to 0.10	0.81
Uric Acid	0.01	-0.09 to 0.11	0.89	0.03	-0.08 to 0.13	0.63
Carbonyls	-0.01	-0.11 to 0.09	0.82	-0.02	-0.12 to 0.08	0.65
F2-Isoprostanes	-0.05	-0.16 to 0.05	0.30	-0.04	-0.15 to 0.06	0.42

Table B (1): Association between having wheeze in the last 12 months and food intake

Food Group	Food Item	Model 1			Model 2			Model 3		
	Per 100g except ^#	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value
Fruits	Orange	0.97	0.02 to 1.04	0.39	0.98	0.92 to 1.04	0.42	0.98	0.92 to 1.04	0.53
	Lemon	0.90	0.60 to 1.35	0.61	0.89	0.58 to 1.37	0.60	0.92	0.60 to 1.42	0.71
	Kiwi ^	1.04	0.81 to 1.35	0.75	1.09	0.83 to 1.42	0.53	1.11	0.85 to 1.45	0.45
	Apple	0.97	0.88 to 1.07	0.57	0.98	0.89 to 1.08	0.63	0.99	0.89 to 1.09	0.80
	Strawberry^	0.83	0.64 to 1.07	0.15	0.86	0.66 to 1.12	0.27	0.87	0.67 to 1.14	0.32
	Mandarin	0.97	0.89 to 1.05	0.45	0.97	0.89 to 1.06	0.47	0.98	0.89 to 1.07	0.60
Vegetables	Beetroot^	1.20	0.92 to 1.55	0.18	1.17	0.89 to 1.53	0.26	1.17	0.89 to 1.53	0.26
	Chard	1.01	0.67 to 1.51	0.98	1.05	0.70 to 1.58	0.82	1.07	0.71 to 1.61	0.75
	S. pepper	1.71	1.00 to 2.94	0.05	1.68	0.97 to 2.92	0.07	1.75	1.00 to 3.06	0.05
	Garlic#	1.00	0.83 to 1.21	0.96	0.97	0.81 to 1.18	0.79	0.99	0.82 to 1.20	0.92
	Onion	0.96	0.79 to 1.17	0.68	0.91	0.74 to 1.12	0.37	0.94	0.76 to 1.16	0.55
	Tomato	0.99	0.94 to 1.04	0.64	0.97	0.92 to 1.02	0.30	0.98	0.93 to 1.04	0.48
	Potato	0.95	0.86 to 1.06	0.37	0.96	0.86 to 1.07	0.43	0.98	0.88 to 1.10	0.76
	Pumpkin	1.09	0.58 to 2.04	0.80	1.01	0.53 to 1.92	0.99	1.15	0.59 to 2.26	0.68
	Carrot	1.07	0.85 to 1.36	0.56	1.08	0.84 to 1.38	0.54	1.13	0.88 to 1.45	0.35
	Avocado	0.97	0.89 to 1.05	0.42	0.96	0.88 to 1.04	0.34	0.98	0.89 to 1.07	0.62
Legumes	Bean	0.94	0.52 to 1.69	0.84	0.82	0.44 to 1.54	0.54	0.89	0.47 to 1.69	0.73
	Lentil	0.71	0.32 to 1.55	0.39	0.65	0.29 to 1.43	0.28	0.68	0.30 to 1.50	0.34
	Chickpeas^	0.95	0.71 to 1.26	0.72	1.05	0.78 to 1.41	0.77	1.06	0.79 to 1.43	0.70
Meats	Red meat	0.85	0.63 to 1.13	0.26	0.87	0.64 to 1.18	0.38	0.91	0.67 to 1.24	0.56
	Chicken	1.01	0.51 to 2.01	0.97	1.17	0.58 to 2.36	0.67	1.20	0.60 to 2.43	0.61
	Pork ribs	0.79	0.39 to 1.61	0.51	0.70	0.33 to 1.49	0.35	0.76	0.35 to 1.67	0.50
	Fish	0.84	0.53 to 1.33	0.46	0.88	0.55 to 1.42	0.61	0.92	0.57 to 1.49	0.75
	Eggs	1.36	0.98 to 1.87	0.06	1.33	0.94 to 1.87	0.11	1.43	1.01 to 2.04	0.05

Continuation Table B (1)

Food Group	Food Item	Model 1			Model 2			Model 3		
	Per 100g except ^ #	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value
Cereals	Bread	0.98	0.93 to 1.04	0.60	0.97	0.91 to 1.04	0.40	1.01	0.91 to 1.11	0.89
	Pasta^	1.14	0.51 to 2.56	0.76	1.17	0.51 to 2.69	0.72	1.22	0.53 to 2.83	0.64
	Rice^	0.72	0.35 to 1.46	0.36	0.77	0.37 to 1.61	0.49	0.80	0.38 to 1.67	0.55
	Cake^	0.77	0.60 to 1.00	0.05	0.87	0.67 to 1.13	0.30	0.89	0.68 to 1.16	0.38
Fatty foods	Oil	2.19	0.71 to 6.75	0.17	1.73	0.54 to 5.55	0.35	2.30	0.68 to 7.75	0.18
	Bacon^	0.91	0.58 to 1.44	0.68	0.87	0.55 to 1.40	0.57	0.91	0.57 to 1.46	0.69
	Sausage^	1.16	0.90 to 1.51	0.26	1.19	0.91 to 1.57	0.21	1.23	0.93 to 1.62	0.15
	Frankfurter	0.69	0.30 to 1.59	0.39	0.68	0.29 to 1.62	0.39	0.78	0.32 to 1.92	0.59
	Ham	1.06	0.68 to 1.64	0.81	1.01	0.63 to 1.61	0.97	1.14	0.70 to 1.87	0.60
	Offal^	1.12	0.84 to 1.50	0.44	1.13	0.84 to 1.53	0.42	1.17	0.86 to 1.59	0.31
	Margarine	0.73	0.32 to 1.68	0.46	0.73	0.32 to 1.67	0.46	0.81	0.35 to 1.86	0.62
	Mayonnaise	1.29	0.41 to 4.04	0.66	1.51	0.46 to 4.92	0.49	2.03	0.58 to 7.02	0.27
Dairy foods	Milk^	0.94	0.73 to 1.22	0.64	0.98	0.75 to 1.28	0.87	0.99	0.75 to 1.29	0.92
	Cheese	0.53	0.28 to 1.02	0.06	0.55	0.29 to 1.03	0.06	0.995	0.988 to 1.00	0.11
Sweets	Sugar	1.37	0.81 to 2.34	0.25	1.36	0.79 to 2.36	0.27	1.50	0.85 to 2.64	0.16
	Jam^	0.85	0.66 to 1.10	0.22	0.93	0.71 to 1.22	0.62	0.95	0.73 to 1.24	0.70
	Honey	1.11	0.80 to 1.54	0.54	1.22	0.87 to 1.71	0.26	1.24	0.88 to 1.74	0.21
	Coke	0.99	0.97 to 1.02	0.71	0.99	0.96 to 1.02	0.55	1.00	0.96 to 1.03	0.77
	Juices^	1.02	0.79 to 1.33	0.86	1.03	0.78 to 1.35	0.83	1.06	0.80 to 1.39	0.70
Beverages	Tea^	1.27	0.98 to 1.66	0.08	1.23	0.93 to 1.61	0.14	1.24	0.94 to 1.63	0.12
	Coffee^	1.23	0.95 to 1.59	0.11	1.17	0.90 to 1.52	0.24	1.17	0.90 to 1.52	0.24
	Red wine^	1.17	0.90 to 1.51	0.23	1.12	0.83 to 1.50	0.47	1.14	0.85 to 1.53	0.39
Other	Salt#	0.98	0.79 to 1.22	0.85	0.91	0.71 to 1.16	0.46	0.92	0.73 to 1.17	0.49

^ Represents a dichotomised variable (does/does not eat that food item once a day)
# Garlic and salt were analysed per 10g increase

Table B (2): Association between having wheeze in the last 12 months and per-doubling increase in nutrient intake

Nutrient group	Nutrient	Model 1			Model 2			Model 3		
		OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value
Energy and macro-nutrients	Energy	0.89	0.73 to 1.08	0.24	0.86	0.68 to 1.08	0.19	0.91	0.50 to 1.67	0.76
	Proteins	0.83	0.67 to 1.01	0.07	0.82	0.65 to 1.03	0.08	0.82	0.59 to 1.15	0.26
	Carbohydrates	0.86	0.71 to 1.05	0.15	0.84	0.67 to 1.05	0.13	0.84	0.55 to 1.31	0.45
	Total lipids	0.96	0.82 to 1.12	0.61	0.95	0.80 to 1.12	0.54	1.12	0.84 to 1.50	0.45
	PUFA	1.03	0.88 to 1.21	0.69	1.01	0.85 to 1.20	0.89	1.18	0.93to 1.49	0.17
	MUFA	0.93	0.81 to 1.07	0.34	0.92	0.79 to 1.07	0.29	0.99	0.78 to 1.27	0.95
	SFA	0.96	0.84 to 1.10	0.56	0.96	0.82 to 1.11	0.56	1.08	0.85 to 1.38	0.51
	Cholesterol	0.98	0.85 to 1.13	0.78	0.99	0.85 to 1.16	0.90	1.07	0.89 to 1.29	0.46
	Omega 6	1.12	0.98 to 1.27	0.09	1.10	0.96 to 1.25	0.17	1.14	0.995 to 1.32	0.06
	Omega 3	1.00	0.93 to 1.08	0.90	1.02	0.94 to 1.10	0.70	1.03	0.95 to 1.12	0.45
	Ratio n6/n3	1.03	0.96 to 1.10	0.40	1.01	0.95 to 1.09	0.69	1.02	0.97 to 1.07	0.58
Vitamins	β–Carotene	1.04	0.93 to 1.15	0.52	1.02	0.92 to 1.14	0.68	1.05	0.94 to 1.18	0.40
	Retinol	0.93	0.85 to 1.02	0.15	0.94	0.85 to 1.03	0.20	0.96	0.86 to 1.06	0.41
	Total vitamin A	1.02	0.90 to 1.15	0.76	1.01	0.89 to 1.14	0.91	1.05	0.91 to 1.20	0.51
	Vitamin B1	0.87	0.73 to 1.05	0.14	0.84	0.68 to 1.03	0.10	0.82	0.56 to 1.20	0.30
	Vitamin B2	0.89	0.74 to 1.07	0.21	0.87	0.71 to 1.08	0.20	0.94	0.64 to 1.37	0.74
	Niacin	0.85	0.70 to 1.04	0.11	0.81	0.64 to 1.01	0.07	0.73	0.47 to 1.13	0.16
	Vitamin B6	0.85	0.70 to 1.04	0.12	0.84	0.67 to 1.04	0.11	0.86	0.63 to 1.16	0.32
	Vitamin B12	0.94	0.86 to 1.04	0.24	0.95	0.86 to 1.06	0.37	0.97	0.87 to 1.09	0.63
	Vitamin C	0.94	0.84 to 1.05	0.28	0.93	0.82 to 1.04	0.21	0.95	0.83 to 1.08	0.42
	Vitamin E	1.07	0.90 to 1.27	0.47	1.04	0.87 to 1.26	0.64	1.24	0.97 to 1.59	0.08
	Folic Acid	0.88	0.74 to 1.04	0.13	0.85	0.70 to 1.02	0.09	0.84	0.63 to 1.14	0.26
	Pantotenic Acid	0.82	0.68 to 1.00	0.05	0.81	0.65 to 0.999	0.05	0.78	0.56 to 1.08	0.13
Minerals	Calcium	**0.80**	**0.68 to 0.95**	**0.01**	**0.80**	**0.67 to 0.96**	**0.02**	0.80	0.64 to 1.00	0.05
	Copper	0.85	0.69 to 1.03	0.10	0.81	0.65 to 1.01	0.07	0.78	0.55 to 1.12	0.18
	Iron	0.87	0.71 to 1.07	0.19	0.83	0.66 to 1.05	0.12	0.82	0.52 to 1.28	0.38
	Magnesium	0.86	0.70 to 1.05	0.13	0.83	0.66 to 1.03	0.09	0.83	0.60 to 1.16	0.28
	Phosphorus	0.82	0.66 to 1.00	0.05	0.80	0.63 to 1.01	0.06	0.76	0.52 to 1.11	0.15
	Potassium	0.85	0.70 to 1.02	0.08	0.82	0.67 to 1.01	0.06	0.83	0.63 to 1.09	0.17
	Selenium	0.87	0.72 to 1.05	0.15	0.85	0.68 to 1.05	0.13	0.87	0.63 to 1.21	0.40
	Sodium	0.85	0.69 to 1.03	0.10	**0.80**	**0.64 to 0.99**	**0.04**	0.82	0.64 to 1.05	0.11
	Zinc	**0.80**	**0.66 to 0.98**	**0.03**	**0.78**	**0.63 to 0.98**	**0.04**	0.75	0.54 to 1.04	0.09

Table B (3): Association between having wheeze in the last 12 months and per-quintile increase of flavonoid intake

Flavonoids	Model 1			Model 2			Model 3		
	OR	95% CI	P value (Trend)	OR	95% CI	P value (Trend)	OR	95% CI	P value (Trend)
Total catechins	1.06	0.97 to 1.16	0.19	1.05	0.95 to 1.15	0.33	1.07	0.97 to 1.18	0.19
Flavonols	0.99	0.91 to 1.08	0.84	0.95	0.87 to 1.05	0.31	0.97	0.88 to 1.07	0.49
Flavones	1.07	0.98 to 1.17	0.12	1.06	0.96 to 1.16	0.25	1.07	0.97 to 1.18	0.16

Table B (4): Association between having wheeze in the last 12 months and per-quintile increase in plasma biomarkers

Biomarkers	Model 1			Model 2		
	OR	95% CI	P value (Trend)	OR	95% CI	P value (Trend)
FRAP	0.90	0.79 to 1.03	0.13	0.87	0.75 to 1.01	0.06
Uric Acid	1.06	0.93 to 1.21	0.40	1.05	0.91 to 1.21	0.49
Carbonyls	0.92	0.81 to 1.05	0.24	0.92	0.81 to 1.05	0.23
F2-Isoprostanes	0.88	0.77 to 1.00	0.05	0.89	0.77 to 1.02	0.09

Table C (1): Association between waking with shortness of breath and food intake

Food Group	Food Item	Model 1			Model 2			Model 3		
	Per 100g except ^#	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value
Fruits	Orange	0.95	0.87 to 1.04	0.30	0.96	0.88 to 1.05	0.35	0.95	0.87 to 1.04	0.31
	Lemon	0.94	0.56 to 1.59	0.83	0.96	0.55 to 1.67	0.89	0.94	0.54 to 1.65	0.84
	Kiwi ^	0.88	0.63 to 1.23	0.45	0.88	0.62 to 1.24	0.47	0.87	0.61 to 1.23	0.43
	Apple	1.00	0.89 to 1.13	0.97	1.00	0.90 to 1.13	0.91	1.00	0.89 to 1.13	1.00
	Strawberry^	0.95	0.68 to 1.33	0.77	0.96	0.69 to 1.36	0.84	0.96	0.68 to 1.35	0.80
	Mandarin	1.04	0.95 to 1.14	0.38	1.04	0.95 to 1.15	0.37	1.04	0.95 to 1.14	0.42
Vegetables	Beetroot^	1.08	0.77 to 1.51	0.64	1.01	0.71 to 1.42	0.97	1.01	0.71 to 1.42	0.97
	Chard	1.29	0.84 to 1.99	0.24	1.31	0.84 to 2.03	0.23	1.29	0.83 to 2.01	0.25
	S. pepper	0.95	0.43 to 2.07	0.89	0.86	0.39 to 1.89	0.71	0.84	0.38 to 1.87	0.67
	Garlic#	0.97	0.62 to 1.52	0.90	0.95	0.59 to 1.62	0.82	0.97	0.61 to 1.55	0.90
	Onion	1.01	0.79 to 1.29	0.93	1.00	0.77 to 1.29	1.00	0.98	0.75 to 1.27	0.87
	Tomato	1.02	0.96 to 1.08	0.56	1.01	0.96 to 1.08	0.63	1.01	0.95 to 1.08	0.78
	Potato	1.01	0.89 to 1.14	0.91	1.03	0.91 to 1.16	0.67	1.01	0.88 to 1.16	0.86
	Pumpkin	0.86	0.36 to 2.02	0.73	0.89	0.38 to 2.09	0.80	0.82	0.34 to 1.98	0.65
	Carrot	1.15	0.86 to 1.52	0.34	1.13	0.85 to 1.51	0.40	1.11	0.82 to 1.50	0.49
	Avocado	1.00	0.90 to 1.10	0.93	1.00	0.90 to 1.10	0.94	0.98	0.88 to 1.09	0.71
Legumes	Bean	1.12	0.54 to 2.33	0.75	1.28	0.59 to 2.78	0.53	1.23	0.56 to 2.71	0.60
	Lentil	0.51	0.17 to 1.54	0.23	0.62	0.21 to 1.83	0.39	0.60	0.20 to 1.78	0.36
	Chickpeas^	0.91	0.63 to 1.32	0.62	1.01	0.69 to 1.48	0.97	1.00	0.68 to 1.47	1.00
Meats	Red meat	1.01	0.71 to 1.44	0.95	1.21	0.84 to 1.75	0.30	1.19	0.82 to 1.74	0.37
	Chicken	1.19	0.50 to 2.83	0.69	1.25	0.51 to 3.02	0.63	1.23	0.50 to 2.98	0.65
	Pork ribs	1.25	0.55 to 2.83	0.59	1.41	0.62 to 3.23	0.41	1.34	0.57 to 3.16	0.50
	Fish	0.998	0.991 to 1.00	0.44	0.90	0.48 to 1.68	0.74	0.87	0.47 to 1.64	0.68
	Eggs	0.99	0.63 to 1.54	0.96	1.06	0.67 to 1.68	0.79	1.03	0.64 to 1.65	0.91

Continuation Table C (1)

Food Group	Food Item	Model 1			Model 2			Model 3		
	Per 100g except ^#	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value
Cereals	Bread	0.96	0.89 to 1.04	0.34	0.99	0.91 to 1.08	0.83	0.94	0.84 to 1.07	0.35
	Pasta^	0.47	0.21 to 1.06	0.07	0.60	0.25 to 1.42	0.25	0.58	0.24 to 1.39	0.22
	Rice^	**0.33**	**0.16 to 0.70**	**0.003**	**0.38**	**0.18 to 0.83**	**0.02**	**0.37**	**0.17 to 0.81**	**0.01***
	Cake^	1.09	0.78 to 1.52	0.60	1.10	0.78 to 1.55	0.57	1.09	0.77 to 1.54	0.62
Fatty foods	Oil	1.09	0.24 to 4.86	0.91	1.24	0.27 to 5.69	0.79	1.08	0.22 to 5.38	0.93
	Bacon^	1.23	0.71 to 2.12	0.46	1.36	0.78 to 2.39	0.28	1.34	0.76 to 2.36	0.32
	Sausage^	0.89	0.63 to 1.24	0.48	0.95	0.68 to 1.34	0.79	0.94	0.66 to 1.33	0.72
	Frankfurter	0.69	0.23 to 2.09	0.52	0.88	0.29 to 2.67	0.83	0.79	0.25 to 2.49	0.69
	Ham	1.19	0.70 to 2.03	0.53	1.42	0.81 to 2.47	0.22	1.38	0.76 to 2.51	0.29
	Offal^	1.13	0.77 to 1.64	0.54	1.11	0.76 to 1.63	0.59	1.09	0.74 to 1.61	0.65
	Margarine	0.82	0.28 to 2.38	0.71	0.86	0.30 to 2.47	0.78	0.80	0.27 to 2.37	0.69
	Mayonnaise	1.12	0.25 to 4.93	0.88	1.56	0.36 to 6.87	0.56	1.38	0.29 to 6.58	0.68
Dairy foods	Milk^	0.90	0.65 to 1.25	0.53	0.97	0.69 to 1.37	0.86	0.96	0.68 to 1.36	0.83
	Cheese	0.92	0.46 to 1.84	0.81	1.01	0.53 to 1.96	0.97	0.95	0.48 to 1.90	0.89
Sweets	Sugar	**2.21**	**1.19 to 4.13**	**0.01**	**2.29**	**1.21 to 4.33**	**0.01**	**2.26**	**1.18 to 4.35**	**0.01***
	Jam^	0.84	0.60 to 1.18	0.33	0.87	0.61 to 1.22	0.42	0.86	0.61 to 1.21	0.38
	Honey	1.03	0.67 to 1.57	0.91	1.09	0.70 to 1.69	0.70	1.08	0.69 to 1.68	0.74
	Coke	0.99	0.95 to 1.03	0.65	1.00	0.96 to 1.04	0.95	1.00	0.95 to 1.04	0.83
	Juices^	1.04	0.74 to 1.46	0.83	1.06	0.74 to 1.50	0.76	1.04	0.73 to 1.48	0.82
Beverages	Tea^	1.32	0.94 to 1.85	0.11	1.34	0.95 to 1.89	0.10	1.33	0.94 to 1.88	0.10
	Coffee^	1.25	0.90 to 1.74	0.19	1.30	0.92 to 1.82	0.13	1.30	0.92 to 1.82	0.13
	Red wine^	1.06	0.76 to 1.48	0.75	1.35	0.92 to 1.98	0.13	1.34	0.91 to 1.97	0.14
Other	Salt#	0.95	0.68 to 1.31	0.75	0.91	0.65 to 1.28	0.59	0.90	0.64 to 1.28	0.57

^ Represents a dichotomised variable (does/does not eat that food item once a day)

Garlic and salt were analysed per 10g increase

* Bonferroni-corrected P-value=0.47

Table C (2): Association between waking with shortness of breath and per-doubling increase in nutrient intake

Nutrient group	Nutrient	Model 1			Model 2			Model 3		
		OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value
Energy and macro-nutrients	Energy	0.93	0.72 to 1.21	0.60	1.07	0.80 to 1.45	0.65	0.89	0.42 to 1.86	0.75
	Proteins	0.81	0.62 to 1.06	0.12	0.91	0.69 to 1.22	0.54	0.72	0.47 to 1.09	0.12
	Carbohydrates	0.94	0.72 to 1.21	0.61	1.06	0.79 to 1.43	0.68	0.94	0.54 to 1.63	0.82
	Total lipids	1.01	0.83 to 1.24	0.91	1.09	0.88 to 1.36	0.42	1.10	0.76 to 1.60	0.62
	PUFA	1.02	0.83 to 1.25	0.86	1.08	0.86 to 1.35	0.50	1.05	0.78 to 1.42	0.73
	MUFA	1.01	0.85 to 1.21	0.89	1.08	0.89 to 1.31	0.42	1.08	0.79 to 1.47	0.62
	SFA	1.01	0.84 to 1.20	0.95	1.09	0.90 to 1.32	0.39	1.09	0.81 to 1.48	0.57
	Cholesterol	0.96	0.80 to 1.14	0.62	1.05	0.86 to 1.28	0.65	1.02	0.80 to 1.29	0.90
	Omega 6	1.02	0.87 to 1.19	0.85	1.04	0.88 to 1.23	0.64	1.03	0.86 to 1.22	0.78
	Omega 3	1.05	0.95 to 1.15	0.37	1.07	0.97 to 1.18	0.19	1.06	0.96 to 1.18	0.24
	Ratio n6/n3	0.97	0.88 to 1.06	0.45	0.95	0.87 to 1.04	0.31	0.96	0.87 to 1.05	0.33
Vitamins	β–Carotene	0.99	0.86 to 1.14	0.89	0.97	0.85 to 1.12	0.72	0.96	0.83 to 1.11	0.56
	Retinol	0.99	0.88 to 1.11	0.85	1.01	0.89 to 1.14	0.87	0.99	0.87 to 1.14	0.93
	Total vitamin A	0.98	0.83 to 1.15	0.80	0.97	0.83 to 1.14	0.73	0.94	0.79 to 1.13	0.52
	Vitamin B1	0.88	0.69 to 1.11	0.28	0.96	0.73 to 1.25	0.75	0.69	0.44 to 1.10	0.12
	Vitamin B2	0.91	0.72 to 1.15	0.43	1.00	0.77 to 1.31	0.99	0.81	0.51 to 1.28	0.36
	Niacin	0.87	0.68 to 1.12	0.29	0.97	0.72 to 1.30	0.83	0.67	0.39 to 1.15	0.14
	Vitamin B6	0.88	0.68 to 1.14	0.35	0.96	0.73 to 1.26	0.76	0.82	0.56 to 1.19	0.30
	Vitamin B12	0.93	0.82 to 1.05	0.24	0.98	0.86 to 1.12	0.73	0.96	0.83 to 1.10	0.56
	Vitamin C	0.98	0.85 to 1.14	0.81	0.97	0.83 to 1.12	0.65	0.94	0.79 to 1.11	0.45
	Vitamin E	1.02	0.81 to 1.28	0.86	1.08	0.85 to 1.38	0.51	1.05	0.77 to 1.44	0.74
	Folic Acid	0.85	0.69 to 1.05	0.14	0.91	0.71 to 1.16	0.45	0.70	0.49 to 1.01	0.06
	Pantotenic Acid	0.85	0.66 to 1.09	0.21	0.92	0.70 to 1.22	0.57	0.72	0.48 to 1.09	0.12
Minerals	Calcium	0.86	0.69 to 1.06	0.16	0.94	0.75 to 1.18	0.61	0.86	0.65 to 1.14	0.29
	Copper	0.86	0.66 to 1.11	0.25	0.93	0.69 to 1.23	0.60	0.70	0.45 to 1.09	0.11
	Iron	0.90	0.69 to 1.16	0.41	1.01	0.75 to 1.36	0.94	0.78	0.45 to 1.36	0.38
	Magnesium	0.89	0.68 to 1.15	0.35	0.96	0.73 to 1.28	0.80	0.80	0.53 to 1.20	0.28
	Phosphorus	0.83	0.63 to 1.08	0.16	0.96	0.71 to 1.29	0.77	0.74	0.46 to 1.19	0.22
	Potassium	0.93	0.73 to 1.19	0.57	0.99	0.77 to 1.28	0.96	0.89	0.63 to 1.26	0.50
	Selenium	0.81	0.64 to 1.03	0.08	0.90	0.68 to 1.18	0.45	0.69	0.46 to 1.02	0.06
	Sodium	0.79	0.62 to 1.03	0.08	0.84	0.64 to 1.10	0.21	0.75	0.55 to 1.03	0.07
	Zinc	0.85	0.66 to 1.10	0.22	0.98	0.73 to 1.31	0.90	0.84	0.55 to 1.27	0.41

Table C (3): Association between waking with shortness of breath and per-quintile increase in flavonoid intake

Flavonoids	Model 1			Model 2			Model 3		
	OR	95% CI	P value (Trend)	OR	95% CI	P value (Trend)	OR	95% CI	P value (Trend)
Total catechins	1.07	0.95 to 1.20	0.28	1.09	0.97 to 1.23	0.15	1.09	0.96 to 1.23	0.18
Flavonols	1.07	0.95 to 1.20	0.28	1.07	0.94 to 1.20	0.30	1.06	0.93 to 1.20	0.37
Flavones	1.02	0.90 to 1.14	0.80	1.00	0.89 to 1.12	0.97	0.99	0.88 to 1.12	0.88

Table C (4): Association between waking with shortness of breath and per-quintile increase in plasma levels of biomarkers

Biomarkers	Model 1			Model 2		
	OR	95% CI	P value (Trend)	OR	95% CI	P value (Trend)
FRAP	0.92	0.77 to 1.09	0.33	0.94	0.78 to 1.14	0.55
Uric Acid	0.90	0.75 to 1.07	0.22	0.92	0.77 to 1.11	0.41
Carbonyls	1.04	0.87 to 1.24	0.65	1.04	0.87 to 1.24	0.66
F2-Isoprostanes	0.93	0.78 to 1.11	0.41	0.96	0.79 to 1.15	0.63

D. ASSOCIATIONS BETWEEN HAVING AT LEAST ONE RESPIRATORY SYMPTOM AND INDEPENDENT VARIABLES

Table D (1): Association between having at least one respiratory symptom and food intake

Food Group	Food Item	Model 1			Model 2			Model 3		
	Per 100g except ^#	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value
Fruits	Orange	0.97	0.92 to 1.03	0.34	0.97	0.92 to 1.03	0.31	0.97	0.92 to 1.03	0.33
	Lemon	1.08	0.76 to 1.53	0.67	1.10	0.76 to 1.58	0.62	1.11	0.77 to 1.59	0.59
	Kiwi ^	1.05	0.83 to 1.33	0.66	1.07	0.84 to 1.36	0.61	1.07	0.84 to 1.37	0.59
	Apple	1.01	0.93 to 1.10	0.73	1.02	0.93 to 1.11	0.71	1.02	0.94 to 1.11	0.66
	Strawberry^	0.80	0.63 to 1.00	0.06	0.82	0.64 to 1.04	0.10	0.82	0.64 to 1.04	0.11
	Mandarin	0.96	0.88 to 1.03	0.24	0.95	0.88 to 1.03	0.22	0.95	0.88 to 1.03	0.23
Vegetables	Beetroot^	1.10	0.87 to 1.38	0.45	1.04	0.81 to 1.32	0.78	1.04	0.81 to 1.32	0.78
	Chard	1.39	0.94 to 2.06	0.10	1.41	0.95 to 2.11	0.09	1.42	0.95 to 2.13	0.09
	S. pepper	**2.10**	**1.15 to 3.82**	**0.02**	**1.97**	**1.07 to 3.62**	**0.03**	**1.99**	**1.08 to 3.69**	**0.03***
	Garlic#	1.01	0.85 to 1.20	0.89	0.88	0.15 to 4.95	0.88	0.99	0.83 to 1.17	0.88
	Onion	0.92	0.77 to 1.10	0.34	0.88	0.73 to 1.06	0.17	0.88	0.72 to 1.06	0.18
	Tomato	1.02	0.98 to 1.07	0.35	1.01	0.97 to 1.06	0.65	1.01	0.97 to 1.06	0.57
	Potato	0.95	0.86 to 1.03	0.22	0.95	0.86 to 1.04	0.29	0.95	0.86 to 1.05	0.30
	Pumpkin	1.13	0.63 to 2.01	0.69	1.06	0.58 to 1.92	0.86	1.09	0.59 to 2.04	0.78
	Carrot	1.13	0.91 to 1.41	0.27	1.12	0.89 to 1.40	0.32	1.14	0.90 to 1.43	0.28
	Avocado	0.99	0.92 to 1.06	0.76	0.98	0.92 to 1.05	0.64	0.99	0.91 to 1.06	0.71
Legumes	Bean	0.82	0.48 to 1.39	0.46	0.74	0.42 to 1.31	0.30	0.74	0.41 to 1.34	0.32
	Lentil	**0.43**	**0.21 to 0.91**	**0.03**	**0.42**	**0.20 to 0.88**	**0.02**	**0.42**	**0.20 to 0.88**	**0.02****
	Chickpeas^	0.86	0.67 to 1.12	0.27	0.95	0.72 to 1.24	0.70	0.95	0.73 to 1.24	0.71
Meats	Red meat	0.84	0.65 to 1.09	0.20	0.93	0.71 to 1.21	0.58	0.93	0.71 to 1.23	0.62
	Chicken	1.06	0.57 to 1.98	0.85	1.17	0.62 to 2.23	0.63	1.18	0.62 to 2.25	0.61
	Pork ribs	0.90	0.48 to 1.66	0.73	0.91	0.48 to 1.73	0.77	0.93	0.48 to 1.80	0.83
	Fish	0.997	0.993 to 1.00	0.20	0.84	0.55 to 1.29	0.42	0.85	0.55 to 1.30	0.45
	Eggs	1.29	0.94 to 1.75	0.11	1.30	0.93 to 1.81	0.13	1.33	0.95 to 1.88	0.10

* Bonferroni-corrected P-value=1.0

** Bonferroni-corrected P-value=0.94

Continuation Table D (1)

Food Group	Food Item	Model 1			Model 2			Model 3		
	Per 100g except ^#	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value
Cereals	Bread	0.98	0.93 to 1.03	0.50	0.99	0.93 to 1.05	0.72	0.99	0.91 to 1.08	0.86
	Pasta^	0.70	0.35 to 1.41	0.32	0.79	0.38 to 1.64	0.53	0.80	0.39 to 1.66	0.55
	Rice^	**0.46**	**0.23 to 0.91**	**0.03**	0.52	0.26 to 1.06	0.07	0.53	0.26 to 1.07	0.08
	Cake^	0.83	0.66 to 1.04	0.11	0.89	0.70 to 1.13	0.34	0.89	0.70 to 1.14	0.36
Fatty foods	Oil	1.49	0.52 to 4.27	0.46	1.42	0.47 to 4.27	0.53	1.55	0.49 to 4.87	0.45
	Bacon^	0.98	0.65 to 1.48	0.94	1.00	0.66 to 1.53	0.99	1.01	0.66 to 1.55	0.96
	Sausage^	1.06	0.83 to 1.34	0.65	1.11	0.87 to 1.42	0.39	1.12	0.88 to 1.44	0.36
	Frankfurter	0.63	0.30 to 1.32	0.22	0.68	0.31 to 1.46	0.32	0.68	0.31 to 1.52	0.35
	Ham	1.13	0.76 to 1.69	0.55	1.18	0.77 to 1.80	0.44	1.24	0.79 to 1.95	0.35
	Offal^	1.00	0.76 to 1.31	1.00	0.98	0.74 to 1.29	0.88	0.99	0.75 to 1.30	0.92
	Margarine	0.68	0.33 to 1.43	0.31	0.67	0.32 to 1.41	0.30	0.68	0.32 to 1.45	0.32
	Mayonnaise	0.95	0.33 to 2.75	0.92	1.12	0.38 to 3.36	0.84	1.20	0.38 to 3.78	0.75
Dairy foods	Milk^	0.96	0.76 to 1.22	0.74	1.01	0.79 to 1.29	0.94	1.01	0.79 to 1.29	0.92
	Cheese	0.71	0.43 to 1.17	0.18	0.73	0.44 to 1.20	0.21	0.73	0.43 to 1.22	0.23
Sweets	Sugar	**1.89**	**1.15 to 3.12**	**0.01**	**1.95**	**1.16 to 3.29**	**0.01**	**2.05**	**1.20 to 3.49**	**0.01***
	Jam^	0.89	0.70 to 1.12	0.31	0.94	0.74 to 1.20	0.63	0.95	0.74 to 1.21	0.66
	Honey	1.18	0.87 to 1.59	0.28	1.27	0.93 to 1.73	0.13	1.28	0.94 to 1.75	0.12
	Coke	0.99	0.97 to 1.02	0.63	1.00	0.97 to 1.02	0.73	1.00	0.97 to 1.03	0.79
	Juices^	0.97	0.77 to 1.23	0.81	0.96	0.75 to 1.23	0.76	0.97	0.75 to 1.24	0.79
Beverages	Tea^	**1.39**	**1.09 to 1.78**	**0.01**	**1.39**	**1.08 to 1.78**	**0.01**	**1.39**	**1.08 to 1.79**	**0.01***
	Coffee^	**1.31**	**1.04 to 1.65**	**0.02**	**1.30**	**1.02 to 1.65**	**0.03**	**1.30**	**1.02 to 1.65**	**0.03****
	Red wine^	1.08	0.86 to 1.37	0.51	1.16	0.88 to 1.52	0.29	1.17	0.89 to 1.53	0.27
Other	Salt#	1.10	0.90 to 1.35	0.35	1.04	0.85 to 1.26	0.74	1.04	0.85 to 1.27	0.72

^ Represents a dichotomised variable (does/does not eat that food item once a day)

Garlic and salt were analysed per 10g increase

* Bonferroni-corrected P-value=0.47 ** = 1.0

Table D (2): Association between having at least one respiratory symptom and per-doubling increase in nutrient intake

Nutrient group	Nutrient	Model 1			Model 2			Model 2		
		OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value
Energy and macro-nutrients	Energy	0.92	0.77 to 1.11	0.39	0.96	0.78 to 1.19	0.73	0.97	0.56 to 1.70	0.92
	Proteins	0.85	0.71 to 1.02	0.09	0.89	0.72 to 1.9	0.26	0.82	0.61 to 1.10	0.19
	Carbohydrates	0.90	0.75 to 1.07	0.23	0.93	0.75 to 1.15	0.49	0.85	0.57 to 1.27	0.43
	Total lipids	0.98	0.85 to 1.13	0.76	1.00	0.86 to 1.17	0.95	1.08	0.83 to 1.41	0.58
	PUFA	1.02	0.88 to 1.18	0.82	1.03	0.88 to 1.20	0.72	1.09	0.89 to 1.34	0.44
	MUFA	0.96	0.85 to 1.09	0.57	0.98	0.86 to 1.13	0.81	1.00	0.80 to 1.25	0.98
	SFA	0.98	0.87 to 1.11	0.76	1.01	0.88 to 1.16	0.85	1.08	0.87 to 1.34	0.49
	Omega 6	1.07	0.96 to 1.20	0.21	1.08	0.96 to 1.21	0.22	1.09	0.97 to 1.24	0.15
	Omega 3	1.01	0.95 to 1.08	0.68	1.03	0.96 to 1.11	0.38	1.04	0.97 to 1.11	0.32
	Cholesterol	1.00	0.88 to 1.14	0.97	1.05	0.91 to 1.21	0.48	1.10	0.93 to 1.30	0.28
	Ratio n6/n3	1.01	0.95 to 1.08	0.69	0.995	0.93 to 1.07	0.88	0.99	0.93 to 1.06	0.86
Vitamins	β–Carotene	1.01	0.92 to 1.11	0.86	0.99	0.90 to 1.09	0.83	0.99	0.89 to 1.10	0.91
	Retinol	0.96	0.88 to 1.04	0.31	0.96	0.88 to 1.05	0.43	0.97	0.88 to 1.06	0.47
	Total vitamin A	1.01	0.90 to 1.13	0.86	0.99	0.88 to 1.11	0.89	0.999	0.88 to 1.13	0.99
	Vitamin B1	0.90	0.76 to 1.06	0.21	0.92	0.76 to 1.11	0.40	0.83	0.58 to 1.18	0.29
	Vitamin B2	0.93	0.79 to 1.10	0.43	0.97	0.80 to 1.17	0.77	0.99	0.71 to 1.40	0.97
	Niacin	0.88	0.74 to 1.05	0.17	0.90	0.73 to 1.11	0.33	0.75	0.50 to 1.13	0.17
	Vitamin B6	0.89	0.74 to 1.06	0.20	0.90	0.74 to 1.09	0.27	0.84	0.64 to 1.10	0.22
	Vitamin B12	0.95	0.87 to 1.04	0.24	0.98	0.89 to 1.07	0.63	0.98	0.89 to 1.08	0.69
	Vitamin C	0.96	0.86 to 1.06	0.42	0.93	0.83 to 1.03	0.18	0.92	0.82 to 1.04	0.18
	Vitamin E	1.05	0.90 to 1.23	0.54	1.06	0.90 to 1.26	0.49	1.15	0.92 to 1.43	0.23
	Folic Acid	0.89	0.76 to 1.04	0.14	0.90	0.76 to 1.08	0.27	0.83	0.63 to 1.09	0.18
	Pantotenic Acid	0.86	0.72 to 1.03	0.09	0.87	0.72 to 1.06	0.16	0.77	0.57 to 1.03	0.08
Minerals	Calcium	**0.81**	**0.69 to 0.94**	**0.01**	**0.83**	**0.70 to 0.98**	**0.03**	**0.78**	**0.64 to 0.95**	**0.01***
	Copper	0.88	0.73 to 1.05	0.15	0.87	0.71 to 1.07	0.20	0.76	0.55 to 1.05	0.10
	Iron	0.90	0.75 to 1.08	0.25	0.92	0.74 to 1.14	0.45	0.83	0.55 to 1.24	0.37
	Magnesium	0.89	0.74 to 1.07	0.19	0.89	0.73 to 1.08	0.24	0.81	0.60 to 1.09	0.16
	Phosphorus	0.83	0.69 to 0.997	0.05	0.87	0.70 to 1.07	0.18	0.74	0.53 to 1.04	0.08
	Potassium	0.89	0.75 to 1.05	0.19	0.89	0.74 to 1.06	0.19	0.83	0.64 to 1.06	0.14
	Selenium	0.87	0.73 to 1.03	0.10	0.91	0.74 to 1.10	0.32	0.84	0.62 to 1.14	0.26
	Sodium	0.86	0.71 to 1.03	0.10	0.85	0.69 to 1.03	0.10	0.82	0.65 to 1.03	0.09
	Zinc	**0.82**	**0.68 to 0.98**	**0.03**	0.85	0.69 to 1.05	0.14	0.75	0.56 to 1.02	0.07

* Bonferroni-corrected P-value=0.47

Table D (3): Association between having at least one respiratory symptom and per-quintile increase in flavonoid intake

Flavonoids	Model 1			Model 2			Model 2		
	OR	95% CI	P value (Trend)	OR	95% CI	P value (Trend)	OR	95% CI	P value (Trend)
Total catechins	1.08	1.00 to 1.18	0.05	1.09	1.00 to 1.19	0.06	**1.10**	**1.00 to 1.20**	**0.04***
Flavonols	1.02	0.94 to 1.11	0.59	1.00	0.92 to 1.09	0.94	1.00	0.92 to 1.09	0.98
Flavones	1.08	0.99 to 1.17	0.08	1.04	0.96 to 1.13	0.34	1.05	0.96 to 1.14	0.31

* Bonferroni-corrected P-value=0.12

Table D (4): Association between having at least one respiratory symptom and per-quintile increase in levels of plasma biomarkers

Biomarkers	Model 1			Model 2		
	OR	95% CI	P value (Trend)	OR	95% CI	P value (Trend)
FRAP	0.89	0.79 to 1.00	0.06	0.89	0.78 to 1.01	0.08
Uric Acid	0.91	0.81 to 1.03	0.14	0.92	0.81 to 1.04	0.19
Carbonyls	0.97	0.86 to 1.09	0.61	0.97	0.86 to 1.09	0.60
F2-Isoprostanes	**0.85**	**0.75 to 0.96**	**0.008**	**0.87**	**0.77 to 0.99**	**0.03***

* Bonferroni-corrected P-value=0.09

APPENDIX 3

Association between FEV_1, and ratio FEV_1/FVC with intake and food items, nutrients, flavonoids and plasma biomarkers

In this appendix information on association between lung function outcomes and individual food items and nutrients is provided. It also includes the analyses for per-quintile intake of flavonoids and plasma biomarkers. As described in appendix 2, there was no evidence for non-linearity in the associations between outcomes and food and nutrient intake, therefore these are presented per 100g, 10g, or dichotomised.

Three statistical models are provided, one with unadjsuted associations, and two with potential confounders. Model two included: age, sex, height (in the case of FEV_1 only), socio-economic confounders, birth weight, and adult BMI. Model 3 added TEI. Bonferroni correction was applied in those test that showed nominal statistical significance.

To examine the association between the outcomes studied and biomarkers as independent variables, only two models were analysed. The rationale for this is that TEI is unlikely to affect levels of biomarkers of oxidative stress or antioxidant status like those included in the current study.

Univariately, intake of several food items was associated with a greater FEV_1. Most of these associations were no longer observed after adjusting for potential confounders, with the exceptions of garlic, onion, juices, and red wine, whose intake remained statistically significantly associated with a greater FEV_1 (Table E (1)). After Bonferroni correction, all the P-values were raised above 0.1.

In relation to nutrients, nearly all of them, with the exception of ratio n6/n3, β–carotene, and total vitamin A, were positively associated with a greater FEV_1. After controlling for confounders only folic acid showed a nominal statistically significant and positive association with this outcome, which was no longer significant after Bonferroni

correction (Table E (2)). There was some evidence of a positive association between intake of total catechins and FEV_1 but this was of borderline statistical significance after controlling for confounders (Table E (3)). A negative association of nominal statistical significance was found between FRAP and FEV_1 in the univariate and multivariable analyses, which remained so after Bonferroni correction. No associations were found for the other biomarkers (Table E (4)).

A decrease in the ratio FEV_1/FVC was associated with a 100 g increase in intake of pumpkin and jam, whereas it was positively associated with intake of pork ribs and cheese (Table F (1)).

A. FEV_1

Table E (1): Association between lung function (best FEV1 (L)) and food items

Food Group	Food Item	Model 1			Model 2			Model 3		
	Per 100g except ^#	b-coeff	95% CI	P value	b-coeff	95% CI	P value	b-coeff	95% CI	P value
Fruits	Orange	0.01	-0.01 to 0.03	0.17	0.003	-0.01 to 0.01	0.60	0.002	-0.01 to 0.01	0.69
	Lemon	**0.21**	**0.09 to 0.32**	**0.001**	0.05	-0.01 to 0.12	0.11	0.05	-0.02 to 0.12	0.13
	Kiwi^	-0.05	-0.13 to 0.03	0.19	0.01	-0.03 to 0.06	0.59	0.01	-0.03 to 0.06	0.64
	Apple	0.01	-0.02 to 0.03	0.70	0.004	-0.01 to 0.02	0.60	0.003	-0.01 to 0.02	0.71
	Strawberries^	**-0.08**	**-0.16 to -0.003**	**0.04**	0.03	-0.02 to 0.07	0.23	0.03	-0.02 to 0.07	0.25
	Mandarin	0.02	-0.01 to 0.04	0.13	0.01	-0.01 to 0.02	0.47	0.004	-0.01 to 0.02	0.55
Vegetables	Beetroot^	**-0.14**	**-0.22 to -0.06**	**<0.001**	0.01	-0.03 to 0.06	0.61	0.01	-0.03 to 0.06	0.61
	Chard	-0.10	-0.22 to 0.02	0.11	0.01	-0.06 to 0.08	0.74	0.01	-0.06 to 0.08	0.79
	S. pepper	-0.10	-0.28 to 0.08	0.28	0.10	0.002 to 0.20	0.05	0.10	-0.001 to 0.20	0.05
	Garlic#	0.03	-0.03 to 0.09	0.33	**0.04**	**0.004 to 0.07**	**0.03**	**0.03**	**0.003 to 0.07**	**0.03***
	Onion	**0.09**	**0.03 to 0.14**	**0.004**	**0.05**	**0.02 to 0.08**	**0.002**	**0.05**	**0.02 to 0.08**	**0.002****
	Tomato	**0.02**	**0.01 to 0.04**	**0.004**	-0.001	-0.01 to 0.01	0.75	-0.003	-0.01 to 0.01	0.56
	Potato	**0.09**	**0.06 to 0.12**	**<0.001**	0.01	-0.01 to 0.02	0.42	0.005	-0.01 to 0.02	0.60
	Pumpkin	0.06	-0.13 to 0.26	0.53	-0.04	-0.15 to 0.07	0.47	-0.06	-0.17 to 0.06	0.32
	Carrot	**-0.10**	**-0.18 to -0.03**	**0.01**	-0.03	-0.08 to 0.007	0.10	-0.04	-0.08 to 0.002	0.06
	Avocado	**0.04**	**0.01 to 0.06**	**0.002**	0.001	-0.01 to 0.01	0.93	-0.002	-0.02 to 0.01	0.78
Legumes	Bean	**0.55**	**0.38 to 0.73**	**<0.001**	0.09	-0.02 to 0.19	0.10	0.08	-0.02 to 0.19	0.13
	Lentil	**0.40**	**0.17 to 0.62**	**0.001**	-0.03	-0.15 to 0.10	0.68	-0.03	-0.16 to 0.10	0.63
	Chickpeas^	**0.12**	**0.04 to 0.21**	**0.01**	-0.05	-0.09 to 0.002	0.06	-0.05	-0.10 to 0.001	0.05
Meats	Red meat	**0.28**	**0.20 to 0.37**	**<0.001**	0.03	-0.02 to 0.07	0.29	0.02	-0.03 to 0.07	0.39
	Chicken	0.16	-0.05 to 0.37	0.13	-0.07	-0.18 to 0.05	0.26	-0.07	-0.19 to 0.05	0.24
	Pork ribs	**0.45**	**0.24 to 0.66**	**<0.001**	0.07	-0.05 to 0.19	0.23	0.06	-0.06 to 0.18	0.31
	Fish	**0.26**	**0.12 to 0.39**	**<0.001**	0.02	-0.05 to 0.10	0.59	0.02	-0.06 to 0.09	0.67
	Eggs	**0.34**	**0.24 to 0.45**	**<0.001**	0.01	-0.05 to 0.08	0.63	0.01	-0.05 to 0.07	0.76

* Bonferroni-corrected P-value=1.0 ** =0.09

Continuation Table E(1)

Food Group	Food Item	Model 1			Model 2			Model 3		
	Per 100g except^#	b-coeff	95% CI	P value	b-coeff	95% CI	P value	b-coeff	95% CI	P value
Cereals	Bread	**0.10**	**0.08 to 0.11**	**<0.001**	-0.004	-0.01 to 0.01	0.52	-0.01	-0.03 to 0.002	0.08
	Pasta^	0.02	-0.22 to 0.27	0.84	-0.03	-0.16 to 0.10	0.66	-0.04	-0.17 to 0.10	0.60
	Rice^	0.01	-0.22 to 0.24	0.94	-0.06	-0.19 to 0.06	0.32	-0.07	-0.20 to 0.06	0.30
	Cake^	-0.07	-0.15 to 0.005	0.07	-0.02	-0.07 to 0.02	0.30	-0.03	-0.07 to 0.02	0.25
Fatty foods	Oil	**0.87**	**0.52 to 1.23**	**<0.001**	0.08	-0.12 to 0.28	0.46	0.06	-0.15 to 0.27	0.59
	Bacon^	**0.30**	**0.17 to 0.44**	**<0.001**	**0.07**	**-0.01 to 0.15**	**0.08**	0.07	-0.01 to 0.14	0.09
	Sausage^	**0.26**	**0.18 to 0.34**	**<0.001**	0.04	-0.01 to 0.08	0.09	0.04	-0.01 to 0.08	0.11
	Frankfurter	**0.63**	**0.39 to 0.87**	**<0.001**	-0.01	-0.15 to 0.12	0.84	-0.03	-0.17 to 0.11	0.66
	Ham	**0.46**	**0.32 to 0.59**	**<0.001**	-0.003	-0.08 to 0.07	0.94	-0.02	-0.10 to 0.07	0.70
	Offal	0.08	-0.01 o 0.17	0.09	0.01	-0.04 to 0.06	0.61	0.01	-0.04 to 0.06	0.69
	Margarine	0.10	-0.14 to 0.33	0.43	-0.001	-0.13 to 0.13	0.99	-0.01	-0.15 to 0.12	0.86
	Mayonnaise	**0.81**	**0.46 to 1.17**	**<0.001**	0.02	-0.18 to 0.22	0.84	-0.005	-0.21 to 0.20	0.96
Dairy foods	Milk^	**0.15**	**0.07 to 0.23**	**<0.001**	-0.01	-0.06 to 0.03	0.63	-0.01	-0.06 to 0.03	0.60
	Cheese	**0.27**	**0.11 to 0.42**	**0.001**	0.04	-0.05 to 0.13	0.38	0.03	-0.06 to 0.12	0.51
Sweets	Sugar	**0.24**	**0.07 to 0.40**	**0.01**	-0.02	-0.11 to 0.07	0.69	-0.03	-0.12 to 0.07	0.56
	Jam^	-0.06	-0.14 to 0.02	0.16	-0.01	-0.06 to 0.03	0.61	-0.01	-0.06 to 0.03	0.56
	Honey^	**0.13**	**0.03 to 0.23**	**0.01**	0.05	-0.01 to 0.11	0.09	0.05	-0.01 to 0.10	0.10
	Coke	**0.03**	**0.02 to 0.04**	**<0.001**	-0.0005	-0.005 to 0.005	0.86	-0.001	-0.006 to 0.004	0.70
	Juices^	**0.11**	**0.03 to 0.19**	**0.01**	**0.06**	**0.01 to 0.30**	**0.01**	**0.06**	**0.01 to 0.10**	**0.01***
Beverages	Tea^	**0.09**	**0.004 to 0.17**	**0.04**	0.04	-0.003 to 0.09	0.069	0.04	-0.004 to 0.09	0.08
	Coffee^	**0.08**	**0.004 to 0.16**	**0.04**	0.03	-0.02 to 0.07	0.25	0.03	-0.02 to 0.07	0.25
	Red wine^	**0.48**	**0.41 to 0.56**	**<0.001**	**0.06**	**0.01 to 0.11**	**0.02**	**0.06**	**0.01 to 0.11**	**0.02****
Other	Salt[#]	0.03	-0.07 to 0.12	0.60	0.002	-3.48 to -2.03	0.00	0.0002	-0.05 to 0.06	0.99

^ Represents a dichotomised variable (does/does not eat that food item once a day)

Garlic and salt were analysed as increase per 10g

* Bonferroni-corrected P-value=0.47 ** =0.94

Table E (2): Association between lung function (best FEV_1 (L)) and per-doubling increase in nutrient intake

Nutrient group	Nutrient	Model 1			Model 2			Model 3		
		b-coeff	95% CI	P value	b-coeff	95% CI	P value	b-coeff	95% CI	P value
Energy and macro-nutrients	Energy	**0.40**	**0.34 to 0.45**	**<0.001**	0.005	-0.03 to 0.04	0.81	-0.07	-0.18 to 0.03	0.16
	Proteins	**0.33**	**0.28 to 0.39**	**<0.001**	-0.001	-0.04 to 0.04	0.97	-0.03	-0.08 to 0.03	0.35
	Carbohydrates	**0.38**	**0.33 to 0.44**	**<0.001**	-0.001	-0.04 to 0.04	0.98	-0.05	-0.13 to 0.02	0.16
	Total lipids	**0.23**	**0.19 to 0.28**	**<0.001**	0.01	-0.02 to 0.03	0.70	-0.01	-0.06 to 0.04	0.63
	PUFA	**0.20**	**0.15 to 0.25**	**<0.001**	0.01	-0.02 to 0.03	0.64	-0.002	-0.04 to 0.04	0.90
	MUFA	**0.20**	**0.16 to 0.24**	**<0.001**	0.01	-0.02 to 0.03	0.66	-0.01	-0.05 to 0.03	0.73
	SFA	**0.22**	**0.18 to 0.26**	**<0.001**	0.01	-0.02 to 0.03	0.64	-0.01	-0.04 to 0.03	0.79
	Omega 6	**0.11**	**0.07 to 0.15**	**<0.001**	0.003	-0.02 to 0.02	0.79	-0.00002	-0.02 to 0.02	1.00
	Omega 3	**0.06**	**0.03 to 0.08**	**<0.001**	0.01	0.0003 to 0.03	0.05	0.01	-0.001 to 0.03	0.06
	Ratio n6/n3	-0.01	-0.03 to 0.01	0.21	-0.01	-0.02 to 0.002	0.10	-0.01	-0.02 to 0.002	0.11
	Cholesterol	**0.26**	**0.22 to 0.30**	**<0.001**	0.01	-0.01 to 0.04	0.28	0.01	-0.02 to 0.04	0.45
Vitamins	β–Carotene	-0.02	-0.06 to 0.01	0.14	-0.003	-0.02 to 0.02	0.76	-0.01	-0.02 to 0.01	0.56
	Retinol	**0.09**	**0.06 to 0.11**	**<0.001**	0.01	-0.01 to 0.02	0.33	0.01	-0.01 to 0.02	0.48
	Total vitamin A	0.002	-0.04 to 0.04	0.92	-0.003	-0.02 to 0.02	0.78	-0.01	-0.03 to 0.02	0.52
	Vitamin B1	**0.33**	**0.28 to 0.39**	**<0.001**	-0.005	-0.04 to 0.03	0.79	-0.06	-0.12 to 0.01	0.07
	Vitamin B2	**0.33**	**0.28 to 0.38**	**<0.001**	0.002	-0.03 to 0.04	0.93	-0.03	-0.10 to 0.03	0.28
	Niacin	**0.38**	**0.32 to 0.44**	**<0.001**	-0.003	-0.04 to 0.03	0.86	-0.07	-0.14 to 0.01	0.08
	Vitamin B6	**0.30**	**0.24 to 0.36**	**<0.001**	0.02	-0.02 to 0.05	0.33	0.01	-0.04 to 0.06	0.60
	Vitamin B12	**0.13**	**0.10 to 0.16**	**<0.001**	0.01	-0.01 to 0.03	0.26	0.01	-0.01 to 0.03	0.37
	Vitamin C	**0.04**	**0.003 to 0.07**	**<0.001**	0.003	-0.02 to 0.02	0.79	0.001	-0.02 to 0.02	0.96
	Vitamin E	**0.22**	**0.17 to 0.27**	**<0.001**	0.01	-0.02 to 0.04	0.62	-0.001	-0.04 to 0.04	0.95
	Folic Acid	**0.30**	**0.25 to 0.35**	**<0.001**	-0.02	-0.05 to 0.02	0.35	**-0.06**	**-0.11 to -0.01**	**0.02***
	Pantotenic Acid	**0.32**	**0.26 to 0.37**	**<0.001**	0.01	-0.03 to 0.04	0.72	-0.01	-0.06 to 0.04	0.68
Minerals	Calcium	**0.25**	**0.20 to 0.30**	**<0.001**	0.01	-0.02 to 0.04	0.42	0.01	-0.03 to 0.04	0.68
	Copper	**0.33**	**0.27 to 0.39**	**<0.001**	0.004	-0.03 to 0.04	0.82	-0.02	-0.08 to 0.04	0.51
	Iron	**0.40**	**0.34 to 0.45**	**<0.001**	0.01	-0.03 to 0.04	0.78	-0.03	-0.11 to 0.04	0.41
	Magnesium	**0.33**	**0.27 to 0.39**	**<0.001**	0.01	-0.03 to 0.05	0.59	-0.003	-0.06 to 0.05	0.91
	Phosphorus	**0.41**	**0.35 to 0.47**	**<0.001**	0.02	-0.02 to 0.05	0.44	0.01	-0.06 to 0.07	0.84
	Potassium	**0.26**	**0.20 to 0.31**	**<0.001**	0.02	-0.02 to 0.05	0.37	0.01	-0.04 to 0.06	0.66
	Selenium	**0.37**	**0.32 to 0.43**	**<0.001**	-0.01	-0.04 to 0.03	0.77	-0.04	-0.09 to 0.02	0.16
	Sodium	**0.27**	**0.21 to 0.33**	**<0.001**	-0.02	-0.06 to 0.02	0.32	-0.04	-0.08 to 0.01	0.10
	Zinc	**0.40**	**0.34 to 0.46**	**<0.001**	0.01	-0.02 to 0.05	0.44	0.01	-0.05 to 0.06	0.81

* Bonferroni-corrected P-value=0.64

Table E (3): Association between lung function (best FEV_1 (L)) and per-quintile increase in flavonoid intake

Flavonoids	Model 1			Model 2			Model 3		
	b-Coeff	95% CI	p value (Trend)	b-Coeff	95% CI	p value (Trend)	b-Coeff	95% CI	p value (Trend)
Total catechins	**0.09**	**0.06 to 0.12**	**<0.001**	**0.02**	**0.001 to 0.03**	**0.04**	0.02	-0.001 to 0.03	0.06
Flavonols	**0.05**	**0.02 to 0.08**	**<0.001**	0.02	-0.0003 to 0.03	0.05	0.01	-0.002 to 0.03	0.08
Flavones	**-0.05**	**-0.07 to –0.02**	**0.001**	0.001	-0.01 to 0.02	0.93	-0.0004	-0.02 to 0.02	0.96

Table E (4): Association between lung function (best $FEV_{1;}$ (L)) and per-quintile increase in plasma levels of biomarkers

Quintiles	Model 1			Model 2		
	b-Coeff	95% CI	p value (Trend)	b-Coeff	95% CI	p value (Trend)
FRAP	**0.10**	**0.07 to 0.14**	**<0.001**	**-0.03**	**-0.05 to -0.01**	**0.01***
Uric acid	**0.11**	**0.07 to 0.15**	**<0.001**	-0.01	-0.03 to 0.02	0.64
Carbonyls	0.03	-0.01 to 0.07	0.09	-0.001	-0.02 to 0.02	0.93
F2-Isoprostanes	**0.05**	**0.01 to 0.09**	**0.01**	0.005	-0.02 to 0.03	0.68

* Bonferroni-corrected P-value=0.04

B. ASSOCIATIONS BETWEEN RATIO FEV_1/FVC AND INDEPENDENT VARIABLES

Table F (1): Association between ratio FEV_1/FVC and food items

Food Group	Food Item	Model 1			Model 2			Model 3		
	Per 100g except^#	b-coeff	95% CI	P value	b-coeff	95% CI	P value	b-coeff	95% CI	P value
Fruits	Orange	0.0002	-0.001 to 0.001	0.75	0.0003	-0.001 to 0.002	0.59	0.0005	-0.001 to 0.002	0.46
	Lemon	-0.006	-0.01 to 0.002	0.16	-0.005	-0.01 to 0.004	0.28	-0.004	-0.01 to 0.004	0.35
	Kiwi^	-0.002	-0.01 to 0.004	0.56	-0.004	-0.01 to 0.001	0.15	-0.004	-0.01 to 0.002	0.18
	Apple	-0.002	-0.004 to 0.0004	0.13	-0.002	-0.004 to 0.0002	0.09	-0.002	-0.004 to 0.0005	0.13
	Strawberries^	0.002	-0.003 to 0.01	0.43	-0.0003	-0.01 to 0.01	0.90	-1.88e-06	-0.01 to 0.01	1.00
	Mandarin	-0.0004	-0.002 to 0.001	0.69	-0.0003	-0.002 to 0.001	0.74	-0.0001	-0.002 to 0.002	0.90
Vegetables	Beetroot^	0.001	-0.005 to 0.01	0.75	0.002	-0.01 to 0.003	0.46	-0.002	-0.01 to 0.003	0.45
	Chard	0.003	-0.01 to 0.01	0.52	-0.0004	-0.01 to 0.01	0.94	0.00002	-0.01 to 0.01	1.00
	S. pepper	-0.005	-0.02 to 0.01	0.47	-0.01	-0.02 to 0.004	0.21	-0.01	-0.02 to 0.01	0.24
	Garlic#	0.0001	-0.004 to 0.004	0.98	-0.0002	-0.004 to 0.004	0.91	0.0001	-0.004 to 0.004	0.96
	Onion	0.0001	-0.004 to 0.004	0.97	0.001	-0.003 to 0.005	0.73	0.001	-0.003 to 0.01	0.49
	Tomato	-0.0003	-0.001 to 0.001	0.53	0.0001	-0.001 to 0.001	0.79	0.0004	-0.001 to 0.001	0.51
	Potato	**-0.002**	**-0.004 to -0.0001**	**0.04**	-0.001	-0.003 to 0.001	0.28	-0.001	-0.003 to 0.001	0.28
	Pumpkin	**-0.02**	**-0.04 to -0.01**	**0.002**	**-0.02**	**-0.03 to -0.01**	**0.01**	**-0.02**	**-0.03 to -0.01**	**0.01***
	Carrot	-0.004	-0.01 to 0.001	0.14	**-0.005**	**-0.01 to -0.0002**	**0.04**	-0.005	-0.01 to 0.0005	0.07
	Avocado	-0.001	-0.002 to 0.001	0.41	2.77e-06	-0.002 to 0.002	1.00	0.001	-0.001 to 0.002	0.57
Legumes	Bean	**-0.01**	**-0.03 to -0.001**	**0.04**	-0.004	-0.02 to 0.01	0.53	-0.003	-0.02 to 0.01	0.70
	Lentil	**-0.02**	**-0.04 to -0.01**	**0.006**	**-0.02**	**-0.03 to 0.0002**	**0.05**	-0.02	-0.03 to 0.001	0.07
	Chickpeas^	0.001	-0.01 to 0.01	0.85	0.001	-0.01 to 0.007	0.82	0.001	-0.01 to 0.01	0.75
Meats	Red meat	**-0.01**	**-0.01 to -0.0002**	**0.04**	-0.002	-0.008 to 0.004	0.50	-0.001	-0.01 to 0.01	0.72
	Chicken	-0.002	-0.02 to 0.01	0.82	0.0004	-0.01 to 0.02	0.96	0.001	-0.01 to 0.02	0.89
	Pork ribs	0.01	-0.01 to 0.02	0.27	**0.02**	**0.003 to 0.03**	**0.02**	**0.02**	**0.01 to 0.04**	**0.01***
	Fish	-0.004	-0.01 to 0.01	0.38	-0.001	-0.01 to 0.01	0.77	-0.001	-0.01 to 0.01	0.92
	Eggs	-0.003	-0.01 to 0.005	0.46	0.003	-0.005 to 0.01	0.48	0.004	-0.004 to 0.01	0.30

* Bonferroni-corrected P-value= 0.47

Continuation Table F (1)

Food Group	Food Item	Model 1			Model 2			Model 3		
	Per 100g except^#	b-coeff	95% CI	P value	b-coeff	95% CI	P value	b-coeff	95% CI	P value
Cereals	Bread	**-0.003**	**-0.004 to -0.001**	**0.00**	**-0.001**	**-0.003 to -0.00005**	**0.04**	-0.002	-0.004 to 0.004	0.12
	Pasta^	-0.01	-0.02 to 0.01	0.53	-0.004	-0.02 to 0.01	0.62	-0.003	-0.02 to 0.01	0.69
	Rice^	-0.002	-0.02 to 0.01	0.78	-0.005	-0.02 to 0.01	0.59	-0.004	-0.02 to 0.01	0.65
	Cake^	-0.001	-0.01 to 0.005	0.81	-0.004	-0.01 to 0.002	0.17	-0.004	-0.01 to 0.002	0.22
Fatty foods	Oil	-0.02	-0.04 to 0.01	0.24	-0.001	-0.03 to 0.02	0.92	0.004	-0.02 to 0.03	0.79
	Bacon^	-0.01	-0.02 to 0.004	0.23	-0.003	-0.01 to 0.01	0.58	-0.002	-0.01 to 0.01	0.69
	Sausage^	-0.005	-0.01 to 0.001	0.08	-0.002	-0.01 to 0.003	0.40	-0.002	-0.01 to 0.004	0.50
	Frankfurter	-0.01	-0.03 to 0.01	0.17	-0.0003	-0.02 to 0.02	0.97	0.003	-0.02 to 0.02	0.75
	Ham	**-0.01**	**-0.02 to -0.003**	**0.01**	-0.01	-0.02 to 0.005	0.29	-0.003	-0.01 to 0.01	0.51
	Offal^	-0.005	-0.01 to 0.002	0.13	-0.004	-0.01 to 0.002	0.17	-0.004	-0.01 to 0.003	0.24
	Margarine	-0.002	-0.02 to 0.02	0.86	0.0002	-0.02 to 0.02	0.98	0.002	-0.01 to 0.02	0.78
	Mayonnaise	-0.02	-0.05 to 0.005	0.11	-0.01	-0.04 to 0.01	0.36	-0.01	-0.03 to 0.02	0.58
Dairy foods	Milk^	-0.002	-0.01 to 0.004	0.49	-0.001	-0.01 to 0.005	0.71	-0.001	-0.01 to 0.005	0.77
	Cheese	0.01	-0.001to 0.02	0.08	**0.01**	**0.002 to 0.02**	**0.02**	**0.02**	**0.005 to 0.03**	**0.01***
Sweets	Sugar	-0.004	-0.02 to 0.01	0.53	-0.0002	-0.01 to 0.01	0.98	0.001	-0.01 to 0.01	0.82
	Jam^	-0.003	-0.01 to 0.002	0.24	**-0.006**	**-0.01 to -0.001**	**0.02**	**-0.006**	**-0.01 to -0.001**	**0.03****
	Honey^	-0.003	-0.01 to 0.004	0.43	-0.004	-0.01 to 0.003	0.30	-0.003	-0.01 to 0.004	0.36
	Coke	-0.0002	-0.001 to 0.0004	0.45	0.0004	-0.0003 to 0.001	0.25	0.0005	-0.0002 to 0.001	0.13
	Juices^	-0.006	-0.01 to -5.95e-06	0.05	-0.005	-0.01 to 0.001	0.10	-0.004	-0.01 to 0.001	0.13
Beverages	Tea^	-0.004	-0.01 to 0.002	0.23	-0.002	-0.01 to 0.004	0.58	-0.001	-0.01 to 0.004	0.64
	Coffee^	-5.71e-06	-0.01 to 0.01	1.00	0.002	-0.004 to 0.01	0.57	0.002	-0.004 to 0.01	0.57
	Red wine^	**-0.01**	**-0.01 to -0.002**	**0.01**	0.0002	-0.01 to 0.01	0.94	0.001	-0.01 to 0.01	0.85
Other	Salt#	-0.0001	-0.01 to 0.01	<0.001	0.001	-0.01 to 0.01	0.86	0.001	-0.01 to 0.01	0.76

^ Represents a dichotomised variable (does/does not eat that food item once a day)

Garlic and salt were analysed as increase per 10g

* Bonferroni-corrected P-value=0.47 ** =1.0

Table F (2): Association between Ratio FEV_1/FVC and per-doubling increase in nutrient intake

Nutrient group	Nutrient	Model 1			Model 2			Model 3		
		b-coeff	95% CI	P value	b-coeff	95% CI	P value	b-coeff	95% CI	P value
Energy and macro-nutrients	Energy	**-0.01**	**-0.01 to –0.05**	**<0.001**	-0.004	-0.01 to 0.001	0.08	-0.01	-0.02 to 0.003	0.14
	Proteins	**-0.007**	**-0.01 –0.003**	**0.001**	-0.004	-0.008 to 0.001	0.13	-0.003	-0.01 to 0.004	0.40
	Carbohydrates	**-0.008**	**-0.01 to –0.004**	**<0.001**	-0.004	-0.009 to 0.001	0.10	-0.005	-0.01 to 0.004	0.27
	Total lipids	**-0.006**	**-0.01 to –0.03**	**<0.001**	-0.003	-0.01 to 0.001	0.09	-0.003	-0.01 to 0.003	0.28
	PUFA	**-0.005**	**-0.008 to –0.002**	**0.004**	-0.002	-0.01 to 0.001	0.19	-0.001	-0.006 to 0.003	0.55
	MUFA	**-0.005**	**-0.01 to –0.002**	**0.001**	-0.003	-0.01 to 0.001	0.11	-0.002	-0.008 to 0.003	0.36
	SFA	**-0.005**	**-0.008 to –0.002**	**<0.001**	-0.003	-0.01 to 0.0004	0.09	-0.003	-0.008 to 0.002	0.26
	Omega 6	**-0.003**	**-0.005 to –0.0001**	**0.04**	-0.001	-0.004 to 0.001	0.35	-0.001	-0.004 to 0.002	0.59
	Omega 3	-0.001	-0.002 to 0.001	0.38	-0.0004	-0.002 to 0.001	0.63	-0.0001	-0.002 to 0.002	0.87
	Ratio n6/n3	-0.0003	-0.002 to 0.001	0.72	-0.0001	-0.002 to 0.001	0.94	-0.0001	-0.002 to 0.001	0.88
	Cholesterol	**-0.005**	**-0.01 to –0.002**	**0.001**	-0.002	-0.005 to 0.001	0.20	-0.001	-0.005 to 0.003	0.50
Vitamins	β–Carotene	-0.002	-0.004 to 0.001	0.14	-0.002	-0.005 to –0.00001	0.05	-0.002	-0.004 to 0.0004	0.10
	Retinol	-0.001	-0.003 to 0.001	0.15	-0.001	-0.003 to 0.001	0.46	-0.0003	-0.002 to 0.002	0.82
	Total vitamin A	-0.002	-0.005 to 0.001	0.16	-0.002	-0.005 to 0.0004	0.10	-0.002	-0.005 to 0.001	0.22
	Vitamin B1	**-0.01**	**-0.01 to –0.005**	**<0.001**	**-0.005**	**-0.009 to –0.0004**	**0.03**	-0.008	-0.02 to –0.0001	0.05
	Vitamin B2	**-0.007**	**-0.01 to –0.005**	**<0.001**	-0.004	-0.008 to 0.0004	0.08	-0.005	-0.01 to 0.003	0.21
	Niacin	**-0.009**	**-0.01 to –0.005**	**<0.001**	**-0.005**	**-0.01 to –0.0002**	**0.04**	-0.009	-0.02 to 0.001	0.07
	Vitamin B6	**-0.007**	**-0.01 to –0.003**	**0.001**	-0.004	-0.008 to 0.001	0.13	-0.003	-0.009 to 0.004	0.38
	Vitamin B12	-0.002	-0.004 to –0.00001	0.05	-0.001	-0.003 to 0.001	0.50	-0.0003	-0.003 to 0.002	0.80
	Vitamin C	-0.001	-0.004 to 0.001	0.28	-0.001	-0.004 to 0.001	0.27	-0.001	-0.004 to 0.002	0.51
	Vitamin E	**-0.006**	**-0.01 to –0.003**	**0.002**	-0.003	-0.007 to 0.0009	0.13	-0.002	-0.007 to 0.003	0.38
	Folic Acid	**-0.008**	**-0.01 to –0.004**	**<0.001**	**-0.005**	**-0.009 to –0.001**	**0.03**	-0.006	-0.01 to 0.00003	0.05
	Pantotenic Acid	**-0.01**	**-0.01 to 0.002**	**0.003**	-0.003	-0.007 to 0.002	0.21	-0.001	-0.008 to 0.005	0.67
Minerals	Calcium	-0.002	-0.01 to 0.001	0.20	0.00003	-0.004 to 0.004	0.99	0.002	-0.002 to 0.007	0.37
	Copper	**-0.01**	**-0.01 to –0.004**	**<0.001**	-0.004	-0.009 to 0.0008	0.11	-0.004	-0.01 to 0.004	0.33
	Iron	**-0.0096**	**-0.01 t0 –0.005**	**<0.001**	-0.005	-0.010 to –0.0001	0.05	-0.008	-0.02 to 0.001	0.08
	Magnesium	**-0.007**	**-0.01 –0.003**	**0.001**	-0.003	-0.008 to 0.001	0.16	-0.002	-0.009 to 0.005	0.51
	Phosphorus	**-0.007**	**-0.01 to –0.003**	**0.001**	-0.003	-0.008 to 0.002	0.30	-0.0003	-0.008 to 0.007	0.94
	Potassium	**-0.005**	**-0.009 to –0.001**	**0.008**	-0.003	-0.007 to 0.002	0.26	-0.001	-0.007 to 0.005	0.73
	Selenium	**-0.009**	**-0.01 to –0.005**	**<0.001**	-0.004	-0.01 to 0.0002	0.06	-0.005	-0.01 to 0.002	0.17
	Sodium	**-0.006**	**-0.01 to –0.002**	**0.004**	-0.003	-0.007 to 0.002	0.24	-0.002	-0.007 to 0.004	0.55
	Zinc	**-0.008**	**-0.01 to –0.004**	**<0.001**	-0.003	-0.008 to 0.001	0.17	-0.002	-0.009 to 0.005	0.53

Table F (3): Association between ratio FEV_1/FVC and per-quintile increase of flavonoid intake

Flavonoids	Model 1			Model 2			Model 3		
	b-coeff	95% CI	p value (Trend)	b-coeff	95% CI	p value (Trend)	b-coeff	95% CI	p value (Trend)
Total catechins	**-0.003**	**-0.01 to –0.001**	**0.001**	-0.002	-0.004 to 0.00001	0.05	-0.002	-0.004 to 0.0003	0.09
Flavonols	-0.001	-0.003 to 0.001	0.17	-0.0004	-0.002 to 0.002	0.71	-0.00002	-0.002 to 0.002	0.99
Flavones	0.0001	-0.002 to 0.002	0.88	-0.0005	-0.002 to 0.001	0.62	-0.0003	-0.002 to 0.002	0.77

Table F (4): Association between ratio FEV_1/FVC and per-quintile increase in plasma levels of biomarkers

Biomarkers	Model 1			Model 2		
	b-coeff	95% CI	p value (Trend)	b-coeff	95% CI	p value (Trend)
FRAP	-0.002	-0.005 to 0.001	0.16	-0.0001	-0.003 to 0.003	0.93
Uric Acid	-0.002	-0.004 to 0.001	0.27	0.0004	-0.002 to 0.003	0.79
Carbonyls	-0.002	-0.005 to 0.001	0.12	-0.002	-0.004 to 0.001	0.26
F2-Isoprostanes	0.0002	-0.003 to 0.003	0.89	0.001	-0.002 to 0.004	0.57